The chemistry of aurodox and related antibiotics

HUBERT MAEHR, MICHAEL LEACH, THOMAS H. WILLIAMS, AND JOHN F. BLOUNT
Chemical Research Department, Hoffmann–La Roche Inc., Nutley, NJ 07110, U.S.A.

Received September 17, 1979

HUBERT MAEHR, MICHAEL LEACH, THOMAS H. WILLIAMS, and JOHN F. BLOUNT. Can. J. Chem. **58**, 501 (1980).

The studies which led to the complete structure elucidation of the antibiotic aurodox, including the chiralities of all eleven asymmetric centers and the *cis–trans* isomerism of all seven exocyclic double bonds, are summarized. Aurodox was correlated stereochemically with mocimycin. Thus, aurodox can be described as *N*-[7-[5(*R*)-[7-[1,2-dihydro-4-hydroxy-1-methyl-2-oxo-3-pyridinyl]-6-methyl-7-oxo-1(*E*),3(*E*),5(*E*)-heptatrienyl]tetrahydro-3(*S*),4(*R*)-dihydroxyfuran-2(*S*)-yl]-6(*S*)-methoxy-5,7(*R*)-dimethyl-2(*E*),4(*E*)-heptadienyl]-α(*S*)-ethyl-5,5-dimethyltetrahydro-2(*R*),3(*R*),4(*R*)-trihydroxy-6(*S*)-[1(*E*),3(*Z*)-pentadienyl]-2*H*-pyran-2-acetamide. Mocimycin, probably identical with kirromycin, is the corresponding *N*-desmethyl homolog.

HUBERT MAEHR, MICHAEL LEACH, THOMAS H. WILLIAMS et JOHN F. BLOUNT. Can. J. Chem. **58**, 501 (1980).

On résume les études qui ont conduit à la résolution complète de la structure de l'antibiotique aurodoxe incluant la chiralité des onze centres asymétriques et l'isomérie *cis–trans* des sept doubles liaisons exocycliques. On a relié stéréochimiquement l'aurodoxe à la mocimycine. Alors, on peut décrire l'aurodoxe comme étant: *N*-[[[[dihydro-1,2 hydroxy-4 méthyl-1 oxo-2 pyridinyl-3]-7 méthyl-6 oxo-7 heptatriényl-1(*E*),3(*E*),5(*E*)]-5(*R*) tétrahydrodihydroxy-3(*S*),4(*R*)furannyl-2(*S*)]-7 méthoxy-6(*S*) diméthyl-5,7 heptadiène-2(*E*),4(*E*)yl] α(*S*)-éthyl diméthyl-5,5 tétrahydro trihydroxy-2(*R*),3(*R*),4(*R*) [pentadiène-1(*E*),3(*Z*)yl]-6(*S*) 2*H* pyranne acétamide-2. La mocimycine probablement identique à la kirromycine est l'homologue *N*-desméthyle correspondant.
[Traduit par le journal]

Introduction

Within the past few years a chemically novel group of narrow-spectrum antibiotics has emerged and already comprises six members. Aurodox[1] (**1***b*) (1–3) and its *N*-desmethyl analog mocimycin (**1***a*) (4–6) were the first members discovered. Kirromycin (7) is most likely identical with mocimycin (8). Efrotomycin (**1***c*) (9, 10) is a glycoside consisting of a disaccharide and aurodox as the aglycone. Dihydromocimycin (**1***e*) (11) and kirrothricin (**1***f*) (12, 13) each contain a 4-hydroxy-5,6-dihydro-2-pyridone ring. Kirrothricin deviates further from the general theme of the other members as it lacks both the central tetrahydrofuran ring and the hydroxy group at position 3 on the terminal tetrahydropyran arrangement. That same 3-deoxy function differentiates heneicomycin (**1***d*) (14) from aurodox. From a biosynthetic viewpoint, therefore, dihydro-**1***d* would have represented a more likely structure for kirrothricin than the proposed structure **1***f*. The structure of azdimycin (15) is as yet unknown.

In addition to great chemical similarity, these antibiotics are all primarily active against a limited number of Gram-positive bacteria, notably bacilli (3, 7, 16, 17), the narrow antibacterial spectrum being ascribed to inefficient uptakes.

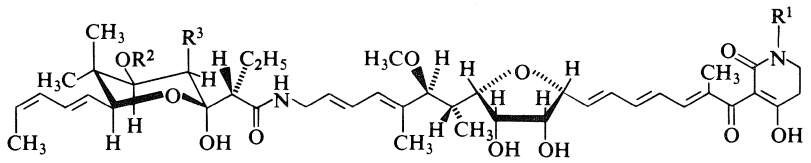
The mechanism of action was studied most intensively with kirromycin (18–26) and was found to involve inhibition of protein synthesis. In prokaryotic cells interaction with the elongation factor Tu was demonstrated. A similar mode of action is probable for all antibiotics of this group and for this reason the cumulative term elfamycins was proposed to emphasize elongation-factor involvement (27). The subsequent observation that elfamycins with EF-Tu specificity include labilomycin (pulvomycin) (26) as a remote structural relative suggests caution in the use of a cumulative term based on mechanism of action alone.

Aurodox (28, 29), mocimycin (30, 31), and efrotomycin (32, 33) are intended for veterinary use, especially as growth-promoting feed additives for farm animals and as chemotherapeutic agents in the control of dysentery caused by Treponema infections. Although mocimycin (**1***a*) and dihydromocimycin (**1***e*) have very similar chemical structures and antimicrobial *in vitro* spectra, only mocimycin promoted growth in animals (11, 34). However, both antibiotics were active against Treponema infections.

Production of these antibiotics appears to be afflicted by generally low fermentation yields; not surprisingly, therefore, aurodox biosynthesis was found to be regulated by feedback inhibition (35) so that production of the other members are likely to be subject to the same phenomenon. Increased yields of aurodox were achieved by aurodox-resistant mutants (36). In addition, a chemical conversion of mocimycin to aurodox was described (27).

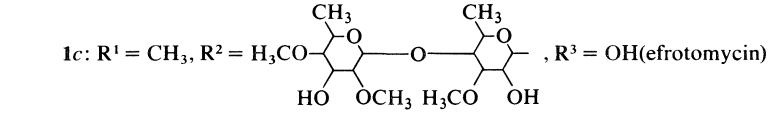
[1]Aurodox is the designation of antibiotic X-5108 adopted by the USAN committee. The previously proposed name "goldinodox" has been withdrawn.

0008-4042/80/050501-26$01.00/0



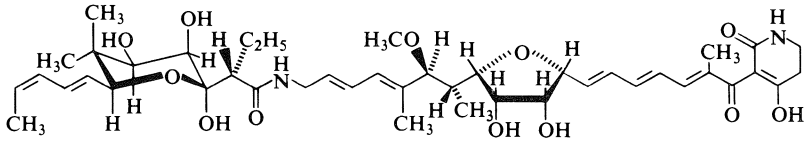
1*a*: $R^1 = R^2 = H$, $R^3 = OH$ (mocimycin, kirromycin)

1*b*: $R^1 = CH_3$, $R^2 = H$, $R^3 = OH$ (aurodox)

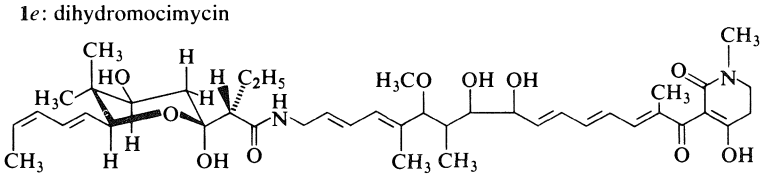


1*c*: $R^1 = CH_3$, $R^2 = H_3CO$–, $R^3 = OH$(efrotomycin)

1*d*: $R^1 = CH_3$, $R^2 = R^3 = H$ (antibiotic A21A, heneicomycin)



1*e*: dihydromocimycin



1*f*: kirrothricin

Aurodox was the first antibiotic of this group to receive a complete structural analysis. In view of the increasing interest in these antibiotics, both as potential commercial agents and biochemical tools, the chemical studies which led to the structure elucidation of aurodox and which were published only in part, are summarized in this paper.

Configuration of Aurodox

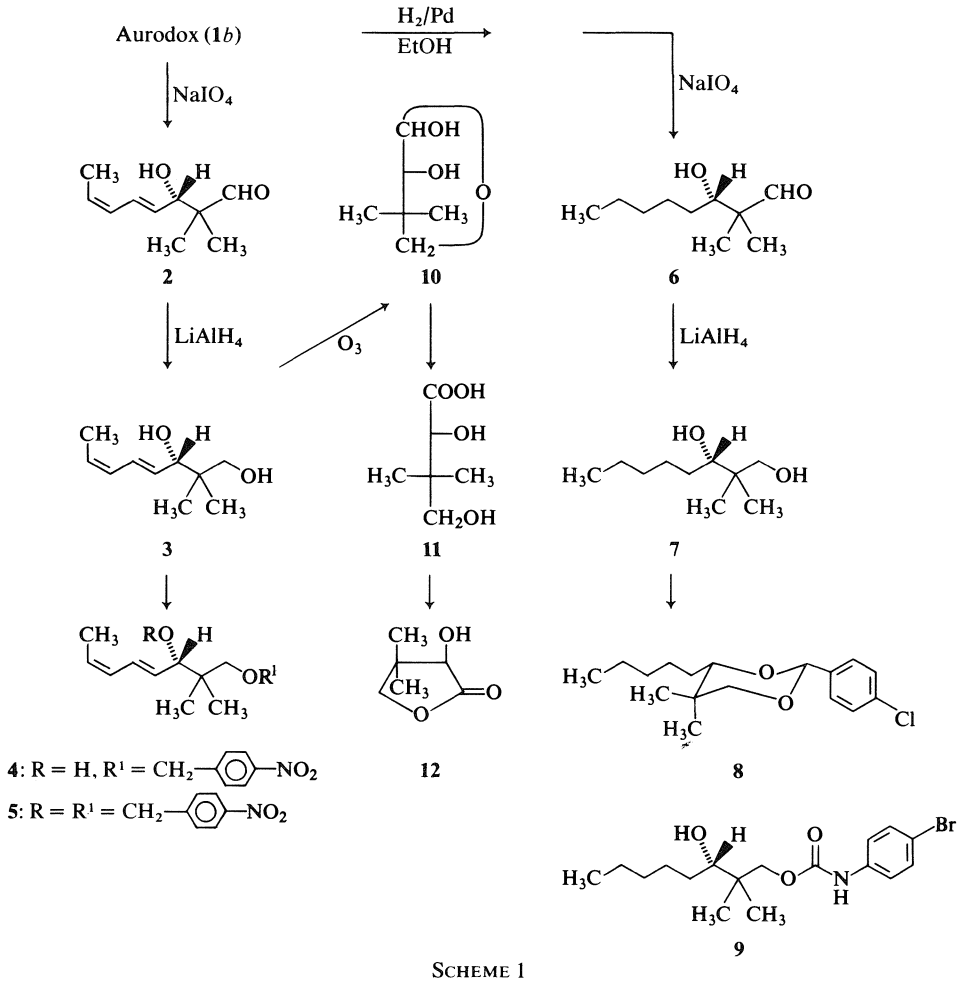
Aurodox was isolated from the fermentation broth of *Streptomyces goldiniensis* by extraction with organic solvents and was precipitated as the sodium salt (3). Although the antibiotic is a yellow, amorphous, monoprotic acid with $pK_a = 6.1$, salts of aurodox could be converted to the acid by extraction with an organic solvent such as dichloromethane even at pH 7. Further purification was achieved by Craig-distribution or chromatography (3).

The acid is soluble in solvents such as ethanol, acetone, chloroform, and ethyl acetate, but is insoluble in water. Alkali salts dissolve in water, pyridine, methanol, and ethanol but are insoluble in acetone and chloroform. Prolonged exposure to light or standing in acidic or basic solutions reduces the antibacterial activity of the antibiotic. Whereas antibiotic titers of the free acid dropped significantly upon storage at room temperature for six months in solid form, no decrease was observed when the sodium salt was stored in the refrigerator for several years.

Elemental analyses of aurodox and alkali salts thereof suggested an empirical formula of $C_{44}H_{62}N_2O_{12} \cdot H_2O$ with $[\alpha]_D$ −82.8° (*c* 0.52, ethanol). Thermoosmotic molecular-weight determination in methanol supported the calculated value of 810. The empirical formula requires 15 units of unsaturation, 7–8 of which were accounted for by rapid hydrogen uptake upon catalytic hydrogenation in ethanol over palladium-on-charcoal.

Aurodox exhibited a characteristic and unique uv spectrum (3) and the ir spectrum indicated polyhydroxy nature. Moreover, aurodox was rapidly oxidized by periodate. Extraction of the oxidation mixture afforded β-hydroxyaldehyde **2** as impure syrup which was reduced with metal hydride and characterized as the resulting diol **3** and its mono- and di-(4-nitrobenzoic) esters **4** and **5** (Scheme 1). The uv maximum at 235 nm observed in **2** prevailed in **3** suggesting the presence of a diene not in conjugation with the aldehyde carbonyl group.

The structure of **2**, including the configuration of the diene, was primarily deduced from the ^1H nmr spectrum of **3** and confirmed by those of **4** and **5** (Fig. 1) (37). Coupling constants of 15 and 11 Hz permitted the assignment of the 4*E* and 6*Z* configurations, respectively, in **2** and the diastereotopic



SCHEME 1

nature of the protons on C1 was evident from the resulting AB pattern in the spectra of **3** and **4**. Coincidentally, however, these protons became isochronous in the corresponding spectrum of the di-nitrobenzoic ester **5** (Fig. 1).

As expected, **1***b* yielded 2,2-dimethyl-3-hydroxyoctanal (**6**) after catalytic hydrogenation and oxidation by periodate. Reduction of **6** with lithium aluminum hydride gave the crystalline diol **7**, which was further characterized as the 2-(4-chlorophenyl)-4-pentyl-5,5-dimethyl-1,3-dioxane (**8**), a colorless liquid obtained by short-path distillation at 3 Torr (bath temperature 145°C), and as the 2,2-dimethyl-1,3-octanediol-1-(4-bromophenyl)carbamate (**9**), obtained as colorless plates with mp 124°C, $[\alpha]_D$ −19.9° (*c* 0.5, dioxane). Ozonolysis of **3** gave D-glycero-3,3-di-*C*-methyltetrose (**10**) which was oxidized to the known D-pantoic acid (**11**) and isolated as D-(−)-pantolactone (**12**) (38, 39) with the same $[\alpha]_D$, and hence with the same chirality, as natural pantothenic acid, thus establishing the chiral center in **2** as 3*S*.

Whereas catalytic hydrogenation of aurodox in ethanol yielded a homogeneous product, a similar reduction in acetic acid caused fragmentation (Scheme 2). One of these degradation products was obtained in crystalline form and ultimately identified as [1*R*-(1β,3α,5α,6α,9α)]-9-ethyl-1,5-dihydroxy-4,4-dimethyl-3-pentyl-2,7-dioxabicyclo[4.3.0]nonan-8-one (**14**) by single-crystal Roentgen-diffraction analysis of its 4-bromobenzoate **15** (Fig. 2) (37). Consonant with the isolation of **14** from aurodox by catalytic hydrogenation in acetic acid, mild treatment of aurodox with acetic acid gave diene **13**, termed goldinono-1,4-lactone-3,7-hemiacetal, derived from the hypothetical parent goldinonic acid **16**[2] and

[2]Trivial names of degradation products were derived from the name of the aurodox-producing Streptomyces goldiniensis.

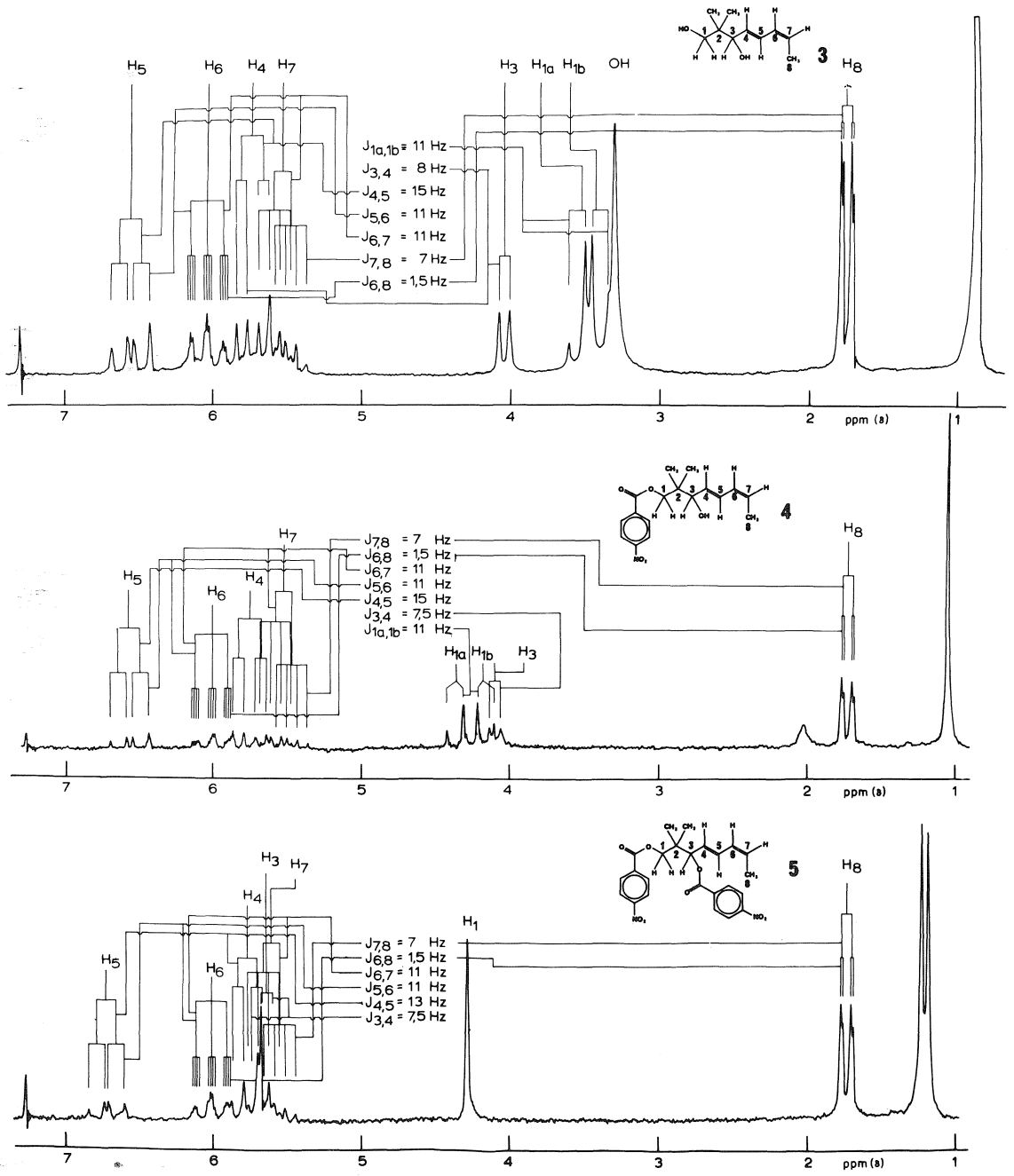
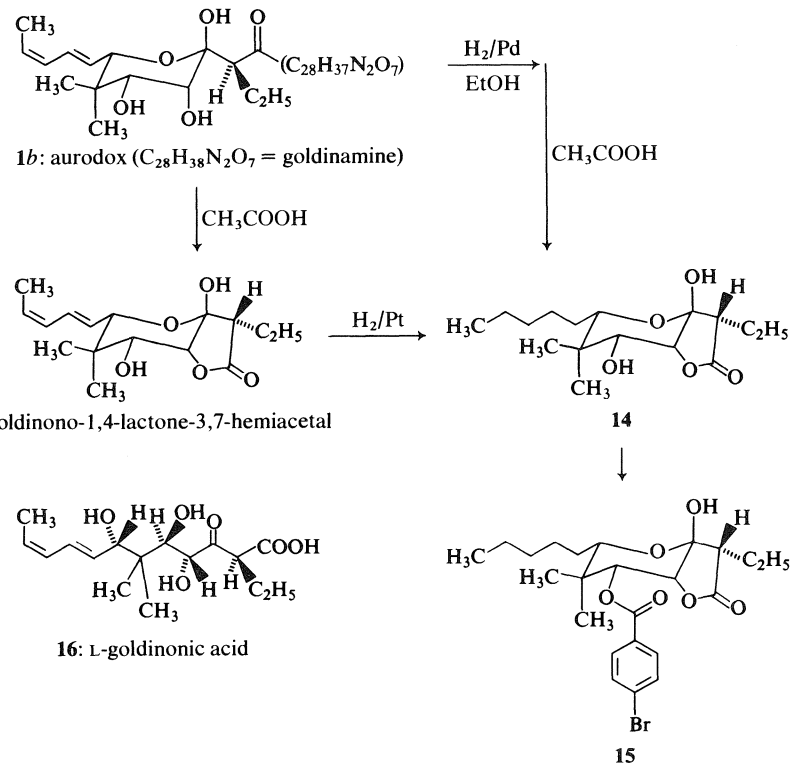


FIG. 1. ^{1}H nmr spectra of **3**, **4**, and **5**.

readily converted to the lactone **14** by catalytic hydrogenation.

Clearly, dienal **2**, obtained by oxidative fission of aurodox by periodate (Scheme 1), is derived from the goldinonic acid moiety, supplying evidence for the (1*E*,3*Z*)-configuration of the pentadienyl side chain in **13** and the presence of a vicinal diol function in aurodox shown in goldinonic acid **16** but absent in goldinono-1,4-lactone-3,7-hemiacetal **13** which is resistant to periodate. Further, the absence of carboxyl-, non-conjugated keto-, and δ-lactone-bands in the ir spectrum of aurodox suggested the goldinonic-acid portion to be arranged in aurodox as the goldinonyl-3,7-hemiacetal form shown in **1***b* (Scheme 2). The facile cleavage of the amide bond with concomitant formation of the δ-



SCHEME 2

lactone function in **13** is explained by anchimeric participation of the axial hydroxyl group of the tetrahydropyran ring resulting in lactone formation and expulsion of the remaining molecular portion $C_{28}H_{38}N_2O_7$.

As potential 1,3-dicarbonyl compounds, **1***b*, **13**, and **14** could conceivably be subject to racemization at the α-carbon center. The nearly quantitative isolation of **14** after hydrogenation and mild acetic acid treatment of **1***b*, however, was interpreted as a good indication that the α(*S*)-configuration is also present in **1***b*.

Catalytic hydrogenation of aurodox sodium salt over palladium-on-charcoal in ethanol followed by hydrolysis in hot water liberated 4-hydroxy-1-methyl-2(1*H*)-pyridone (**19**) (40), characterized as the formaldehyde condensation product **20** (Scheme 3), whereas unreduced aurodox was resistant to hydrolysis under the same conditions. With a pK_a of 6.67, **19** was suspected to be responsible for the acidic nature of the antibiotic and to be linked via C3 in the aurodox molecule, for the two protons at C5 and C6 in **19** were readily identified as a characteristic AB pattern in the ^1H nmr spectrum of **1***b*. Further, the liberation of **19** by hydrolysis after catalytic reduction of **1***b* suggested linkage to a carbonyl group; reduction of the carbonyl group generates an aldol-type arrangement severable by mild hydrolysis.

To exploit the mild fragmentation reaction by acetic acid, which had led to **13** as the only crystalline product together with numerous other components, aurodox sodium salt was reacted with 4-bromobenzyl bromide to yield aurodox(4-bromobenzyl) ether **18**. Subsequent acetic acid treatment of **18** and analysis of the reaction mixture were hoped to be facilitated by the bromobenzyl group providing ready recognizability, blockage of one reactive site, and a heavy atom for possible crystallographic analysis of **18** or appropriate fragments thereof. Aurodox derivative **18**, however, was an amorphous but homogeneous compound and the resulting reaction mixture with acetic acid fortunately contained only two major components, namely **13** and a new, larger fragment, termed goldinamine(4-bromobenzyl) ether (**22**), easily separated from each other by Craig-distribution or chromatography on silicic acid. Goldinamine(4-bromobenzyl) ether contained the uv-chromophore of aurodox with the characteristic maximum at 325 nm and proved to be an amorphous amine, generating salts with acids, a yellowish color with ninhydrin, and *N*-(2,4-dinitrophenyl) derivative **26** with 1-fluoro-2,4-dinitrobenzene.

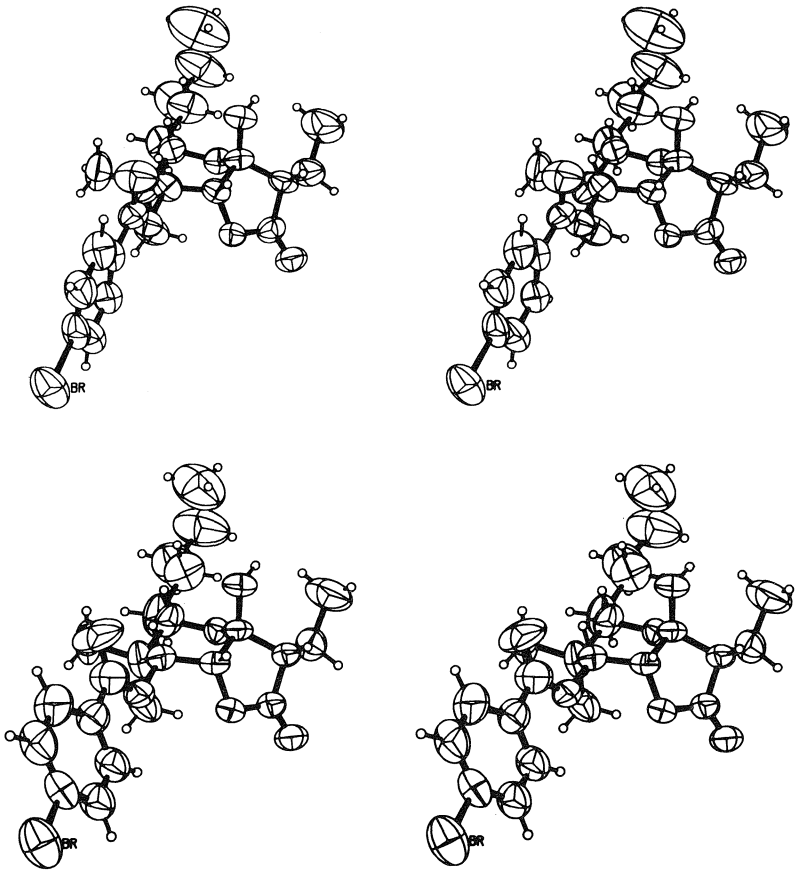


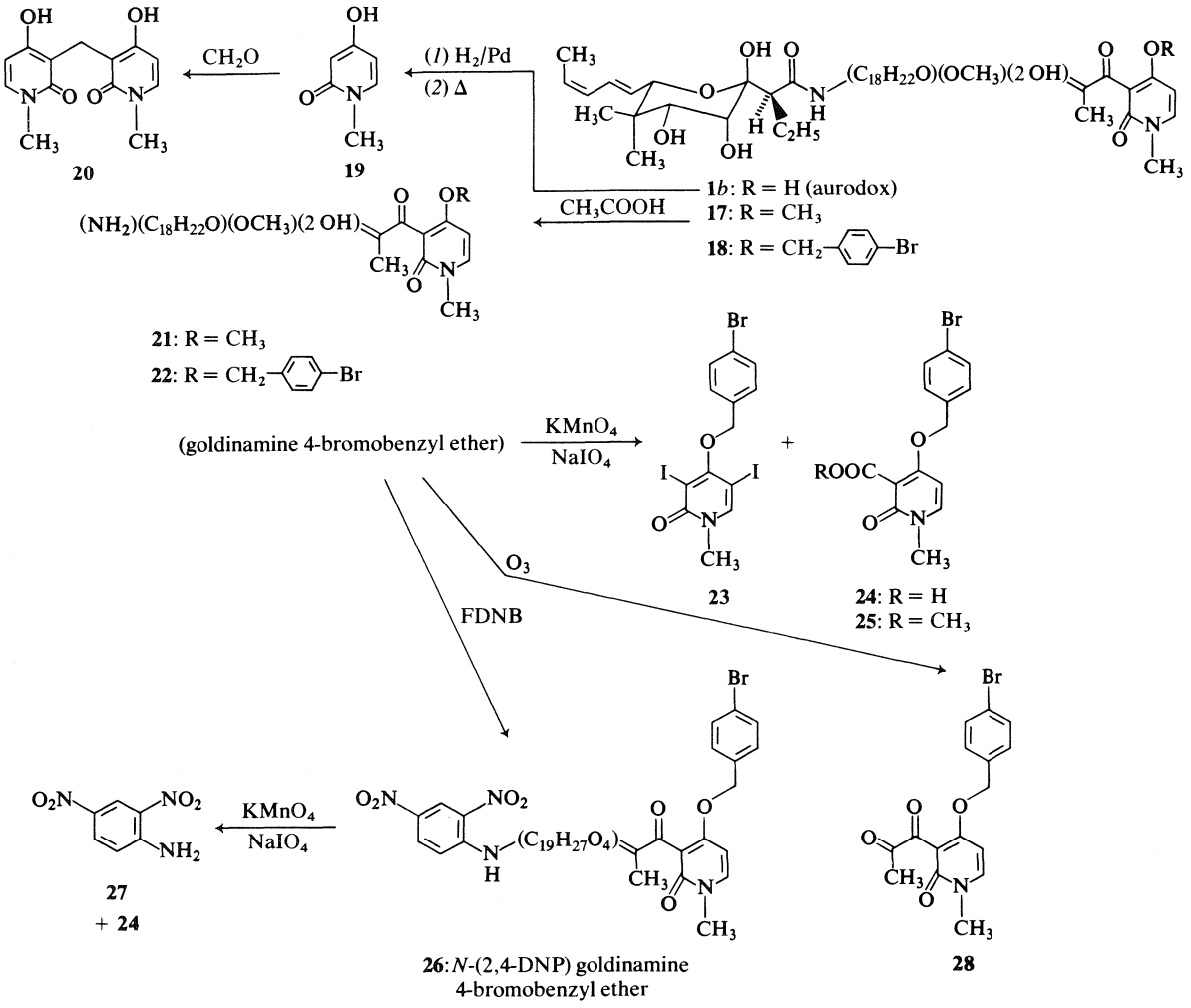
FIG. 2. Stereodrawing showing the absolute configuration of **15** (37) and the conformations of the two independent molecules found in the crystal. The thermal ellipsoids are scaled to the 50% probability level. The hydrogen atoms are shown as spheres of arbitrary size at their calculated positions; they were not included in the structure analysis.

The absence of an amino function in the intact antibiotic suggested goldinonic acid 3,7-hemiacetal to be linked to goldinamine through an amide bond as shown in Scheme 3.

The elemental composition of goldinamine, $C_{28}H_{38}N_2O_7$, was originally estimated by analyses of a number of salts and amino derivatives of **22**, none of which were obtainable in crystalline form.

Oxidation of **22** with a mixture of potassium permanganate and sodium metaperiodate gave crystalline 4-(4-bromobenzyloxy)-1-methyl-1,2-dihydro-2-oxopyridine-3-carboxylic acid (**24**), further characterized as methyl ester **25**, and slightly yellow needles of an artifact, identified as 4-(4-bromobenzyloxy)-3,5-diiodo-1-methyl-2(1*H*)-pyridone (**23**). Ozonolysis of **22** in methanol deposited crystalline 4-(4-bromobenzyloxy)-1-methyl-3-pyruvoyl-2(1*H*)-pyridone (**28**), whereas oxidation of goldinamine derivative **26** with potassium permanganate and sodium metaperiodate afforded **24** as well as 2,4-dinitrobenzeneamine (**27**).

N-Acetylgoldinamine(4-bromobenzyl) ether (*N*-acetyl-**22**) formed a di-*O*-acetyl derivative with acetic anhydride and pyridine, a cyclic carbonate with ethyl chloroformate, an *O*-isopropylidene derivative with acetone and a dialkoxyphenylborane with benzeneboronic acid indicating two vicinal non-tertiary hydroxyl groups. A methoxy group evident from the ^{1}H nmr spectra of **1***b* and **22** led to **1***b* (Scheme 3) as partial structure for aurodox. Indeed, **22** reacted rapidly with sodium metaperiodate liberating 8-[4-(4-bromobenzyloxy)-1,2-dihydro-1-methyl-2-oxo-3-pyridyl]-7-methyl-8-oxo-2,4,6-octatrienoic acid (**31**), obtained in crystalline form, syrupy 8-amino-3-methoxy-2,4-dimethyl-4,6-octadienal (**33**), and two equivalents of formic acid (Scheme 4) (41). Both trienoic acids **29** and **31**, the former accessible by an analogous oxidation of goldinamine methyl ether (**21**), contained the long-wave uv maximum of aurodox and of goldinamine derivatives **21** and **22**, and were readily converted to methyl esters **30** and **32** with diazo-



SCHEME 3

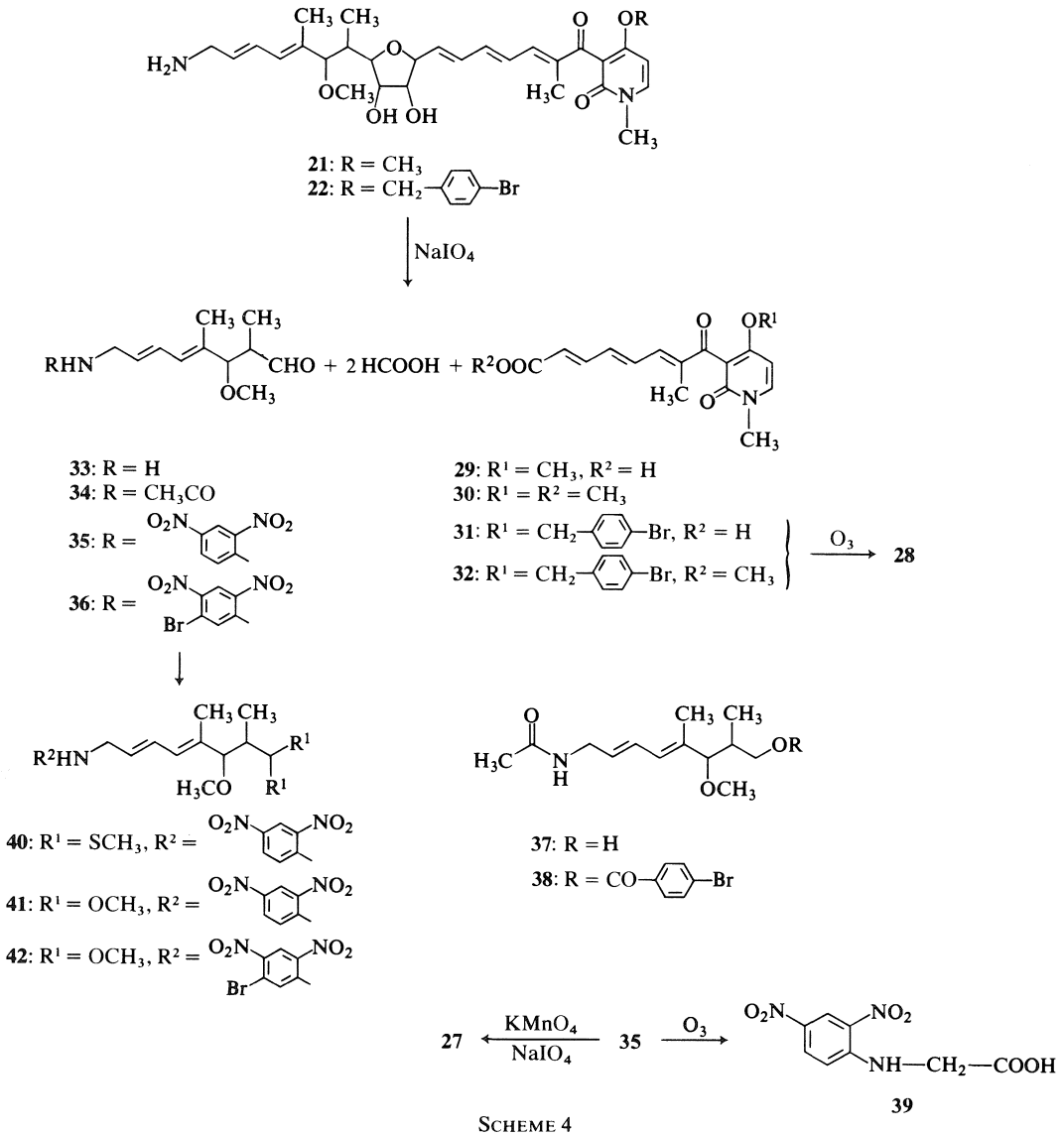
methane. Both **31** and **32** were degraded by ozonolysis to **28** previously obtained from **22**.

With the exception of the double bond at position 6, the configuration of trienoic acid **31** could be deduced from the ^1H nmr spectrum as shown in Fig. 3.

The olefin protons exhibited a five-spin system consisting of one doublet at δ 5.98 ($J_{2,3}$ = 15 Hz) attributed to H2, one doublet of quartets at δ 6.81 ($J_{5,6}$ = 11 and $J_{6,Me}$ = 1 Hz) attributed to H6, and three doublets of doublets. The doublet of doublets at lowest field at δ 7.32 was assigned to H3, since it possesses the same splitting ($J_{2,3}$ = 15 Hz) present in the H2 doublet. Both H4 and H5 exhibited doublets of doublets with identical coupling constants, but the doublet of doublets at δ 7.13 was assigned to H5 on the basis of the distortion of the H6 signal. Thus, it was possible to assign the (2*E*,4*E*)-configuration on the basis of the two large coupling constants $J_{2,3}$ and $J_{4,5}$.

The (6*E*)-configuration was established by single-crystal Roentgen-diffraction analysis (41) of **31**. The crystal was obtained as a 1:1 adduct with chloroform. As seen in Fig. 4, the *xy*-plane bisecting the π-orbital at carbonyl carbon C8 is not parallel with the corresponding plane bisecting the π-orbital of the pyridone ring and renders the molecule dissymmetric. The crystal is centrosymmetric with both enantiomeric conformations present.

The dienal **33** (Scheme 4) was isolated as the syrupy *N*-acetyl and *N*-(2,4-dinitrophenyl) derivatives **34** and **35**, respectively, and yielded the elemental composition of the parent substance **33** as $C_{11}H_{19}NO_2$ by mass spectrometry. Oxidation of **35** with potassium permanganate and sodium metaperiodate gave 2,4-dinitrobenzeneamine. With



SCHEME 4

ozone, followed by oxidative work-up, *N*-(2,4-dinitrophenyl)-glycine (**39**) was obtained from **35** and indicated the presence of an allylic amine in **33**. Sodium borohydride reduced **34** to the dienol **37** with the unaltered uv maximum at 240 nm, originally observed in both **33** and its *N*-acetyl derivative **34**, implying the presence of a 2,4-pentadienylamine in **33** without conjugation of the diene with the aldehyde group. The structure of **35** as shown in Fig. 5, with the exception of the double-bond configuration at C4, was readily derived from the ^{1}H nmr spectrum.

The *threo*-configuration in **33** was originally deduced from the intensity ratio of free vs. bonded hydroxyl stretching-absorptions in the ir spectrum of alcohol **37** (42). The observed value of 0.26 was in good agreement with the reported value of 0.15 for *threo*-3-ethoxy-2,4-dimethyl-4-hexenol (43), permitting hydrogen bonding in a stereochemically less crowded conformation as compared to the less stable hydrogen-bonded arrangement of the *erythro* isomer with a reported value of 1.4 (Fig. 6). Further, inspection of the rather diagnostic ^{1}H nmr chemical shift of the methyl group at C2 in **37** (δ 0.66) was also in good agreement with that of *threo*-3-ethoxy-2,4-dimethyl-4-hexenol (δ 0.60), whereas two synaxial interactions between a methyl group and an unshared electron pair on oxygen cause a significant paramagnetic shift of the methyl group in the *erythro* isomer (δ 0.90) (43).

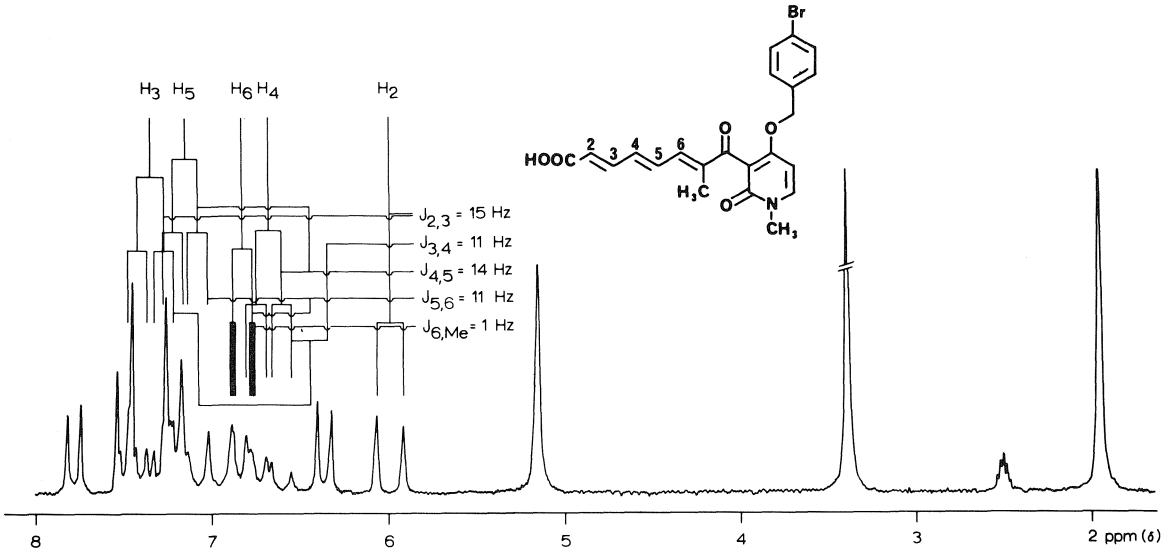


FIG. 3. ^{1}H nmr spectrum of **31**.

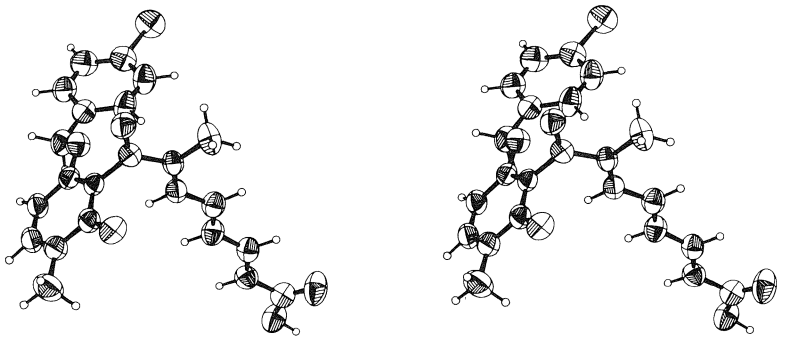


FIG. 4. Stereodrawing of one of the two enantiomeric conformations of **31** present in the crystal.

Attempts to crystallize **37** or esters such as **38** failed. Of a number of acetals prepared only **40** and **41** were crystalline. Of the two only **41** was suitable for a single-crystal Roentgen-diffraction analysis which confirmed the previously assigned *threo* configuration, established the all-*trans* isomerism, but, in the absence of a heavy atom, left the chirality undetermined. Introducing an anomalous scatterer while maintaining all other structural features of the one crystallographically suitable acetal **41** was accomplished with a new reagent, 1-bromo-5-fluoro-2,4-dinitrobenzene (42). Accordingly, [*threo*-(4*E*,6*E*)]-8-[(5-bromo-2,4-dinitrophenyl)-amino]-3-methoxy-2,4-dimethyl-4,6-octadienal (**36**) was prepared from **33** and converted to the crystalline acetal **42** which was indeed suitable for crystallographic analysis (42) establishing the absolute configuration shown in Fig. 7 (bottom). The crystal structure of **41** (41) is shown on top of Fig. 7 for comparison.

As illustrated in Scheme 4, periodate cleaves goldinamine derivatives **21** and **22** in the center, each liberating two moles of formic acid as well as dienal **33** and a trienoic acid as the two terminal fragments. The identification of this center as a 2,5-disubstituted 3,4-dihydroxytetrahydrofuran ring was first accomplished by analysis of the ^{1}H nmr spectra of goldinamine derivatives **26** and **43** (Fig. 8) (41). There were four methine protons, H2′–H5′, attached to sp^3-hybridized carbons in *N*-(2,4-dinitrophenyl) goldinamine (4-bromobenzyl) ether (**26**) which were not part of either degradation products **31** or **33**. Conversion of **26** to the di-*O*-acetyl derivative **43** shifted protons H2′–H5′ paramagnetically, rendering them recognizable as four dd sets and part of a multiple-spin system. Subjected to the more intensive paramagnetic shifts, protons H3′ and H4′ were assigned to carbons bearing acetoxy groups in vicinal relationship based on a common coupling constant

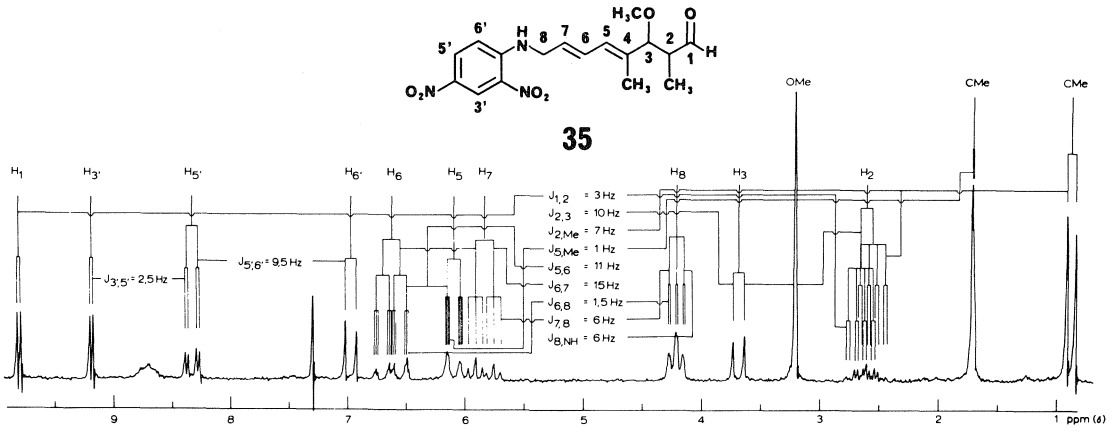
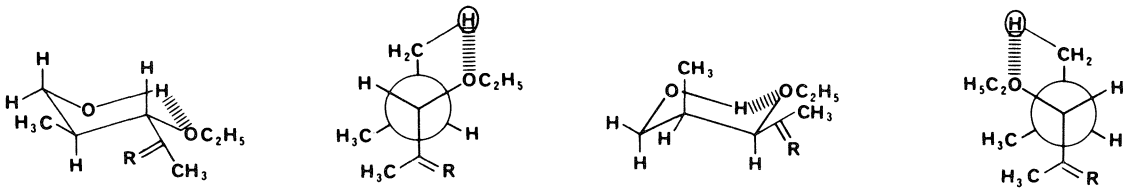


FIG. 5. ^{1}H nmr spectrum of **35**.



threo - 3-ethoxy-2,4-dimethyl-4-hexenol

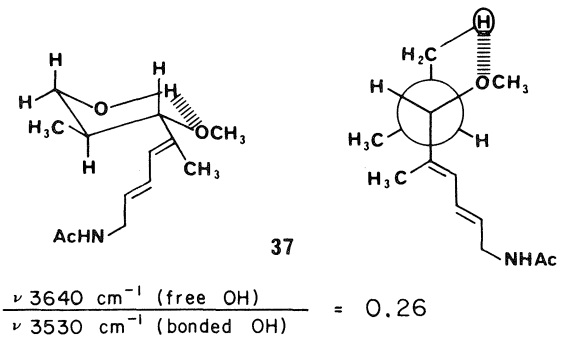
$\frac{\nu\ 3638\ cm^{-1}\ (free\ OH)}{\nu\ 3518\ cm^{-1}\ (bonded\ OH)} = 0.15$

$\delta^{CDCl_3}_{TMS}$ 0.60 ($\underline{CH_3}$–CH)

erythro - 3-ethoxy-2,4-dimethyl-4-hexenol

$\frac{\nu\ 3638\ cm^{-1}\ (free\ OH)}{\nu\ 3520\ cm^{-1}\ (bonded\ OH)} = 1.4$

$\delta^{CDCl_3}_{TMS}$ 0.90 ($\underline{CH_3}$–CH)



37

$\frac{\nu\ 3640\ cm^{-1}\ (free\ OH)}{\nu\ 3530\ cm^{-1}\ (bonded\ OH)} = 0.26$

$\delta^{CDCl_3}_{TMS}$ 0.66 ($\underline{CH_3}$–CH)

FIG. 6. Determination of the *threo* configuration in **37**.

of 5 Hz. Coupling constants $J_{2',3'} = 7$ and $J_{4',5'} = 4$ Hz established the connection C2′—C3′—C4′—C5′. Irradiations indicated by bold arrows (Fig. 9), at the proton frequencies of H1 and H2′ in **43**, resulted in the expected multiplicity changes as depicted; the surviving coupling constants are also shown. The resulting side-chain assignments to positions 2′ and 5′ in **43** could be confirmed by analysis of the ^{1}H nmr spectrum of the *O*-isopropylidene-*N*-(2,4-dinitrophenyl)-goldinamine(4-bromobenzyl) ether (**44**) exhibiting a new set of coupling constants; concordantly, irradiations at the proton frequencies of H4′ and H6′ in **44** led to the spectral simplifications illustrated in Fig. 9 (8).

To ascertain the exact elemental composition of

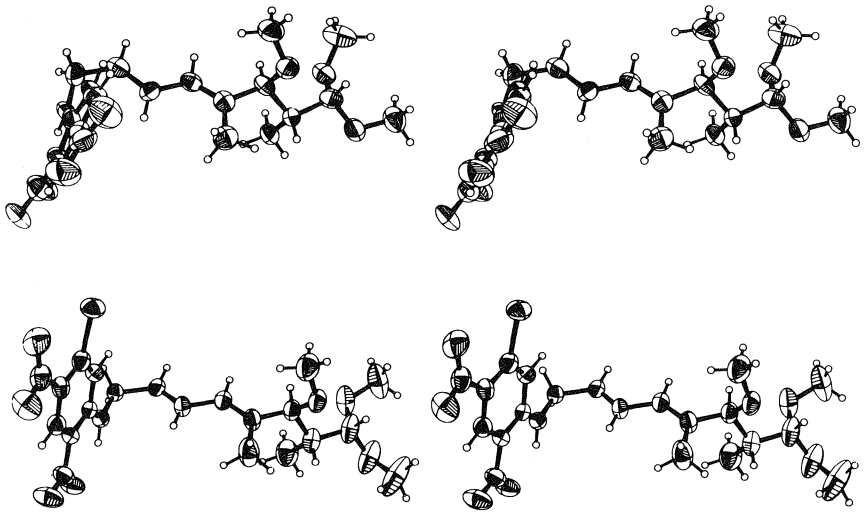


FIG. 7. Stereodrawings showing the conformation and absolute configuration of **41** (upper) and **42** (lower). The thermal ellipsoids are scaled to 50% probability level. The hydrogen atoms are shown as spheres of arbitrary size.

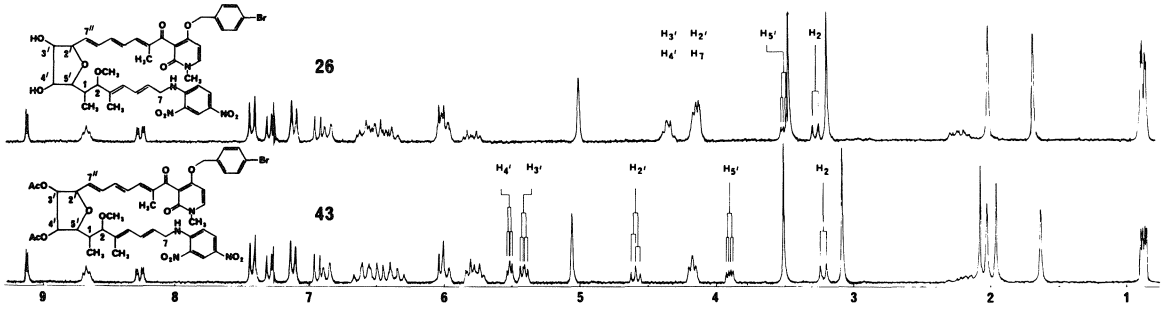


FIG. 8. 220 MHz nmr spectra of goldinamine derivatives **26** and **43**.

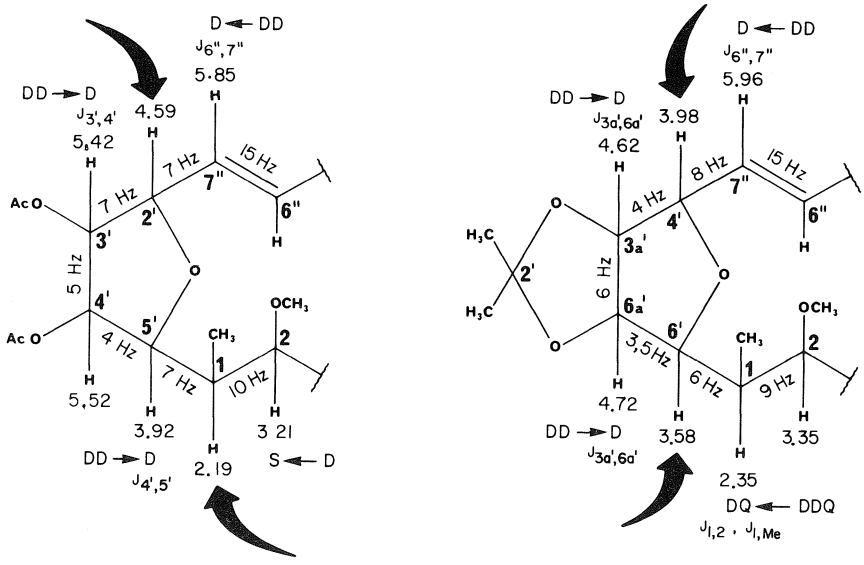
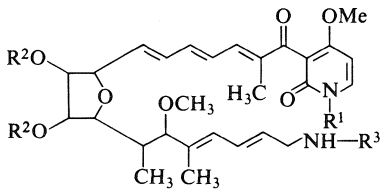


FIG. 9. 220 MHz nmr data characterizing the tetrahydrofuran moiety of goldinamine derivatives **43** and **44**.

goldinamine, various derivatives were investigated by electron-impact mass spectrometry, but useful spectra were only obtained after replacement of the 4-bromobenzyl group in **22** by a methyl substituent. The resulting **21** was readily prepared by acetic acid treatment of aurodox methyl ether (**17**) (Scheme 3) and converted to goldinamine derivatives **47–50** whose mass spectra exhibited molecular ions, with the exception of **48** which gave *m/e* 588 ($M - CO_2 - H_2O$) as the highest observable mass peak. Molecular formulas of **21** and **47–50**, and hence that of goldinamine itself, could be deduced unequivocally on the basis of the low-resolution mass spectra of **47–50** and the known chemistry of the periodate-oxidation products derived from **22** (Scheme 4), but were further confirmed by high-resolution mass spectra of **49** and **50**.



21: R^1 = Me, R^2 = R^3 = H	$C_{29}H_{40}N_2O_7$ (528)
45: R^1 = R^2 = R^3 = H	$C_{28}H_{38}N_2O_7$ (514)
46: R^1 = R^2 = H, R^3 = $COCF_3$	$C_{30}H_{37}F_3N_2O_8$ (610)
47: R^1 = Me, R^2 = H, R^3 = $COCF_3$	$C_{31}H_{39}F_3N_2O_8$ (624)
48: R^1 = Me, $2R^2$ = CO, R^3 = $COCF_3$	$C_{32}H_{37}F_3N_2O_8$ (650)
49: R^1 = Me, R^2 = H, R^3 = Ac	$C_{31}H_{42}N_2O_8$ (570)
50: R^1 = R^2 = Me, R^3 = Ac	$C_{33}H_{46}N_2O_8$ (598)

The tetrahydrofuran structure in goldinamine and aurodox does not remain unexplained by the surprising periodate-oxidation products shown in Scheme 4. Hesse and Mix had demonstrated that carbon–carbon double bonds are cleavable by periodate if at least one carbon is substituted by a free hydroxyl group and if the double bond is in conjugation with a carbonyl group (44). The oxidation of **22** by periodate could thus be postulated to proceed as depicted in Scheme 5 without intention to differentiate between homolytic and heterolytic processes or to show actual hydration states of the iodine compounds. After initial glycol cleavage one of the formyl groups enolizes to become part of a conjugated system; the new double bond conforms to the Hesse–Mix criteria of cleavability by periodate as it contains a hydroxyl group and is in conjugation with a carbonyl group, although only in a hexatrienylogous fashion. A molecule of periodate, it is now implied, adds across the enolic double bond, followed by decomposition of the resulting cyclic iodic ester to liberate formic acid, hypothetical iodous acid, and an ester which yields **31** as well as an α-hydroxyaldehyde upon hydrolysis, the latter being subject to further oxidation with periodate to afford a second mole of formic acid and dienal **33**. The possible intermediacy of iodous acid is supported by the transient appearance of free iodine by disproportionation (41).

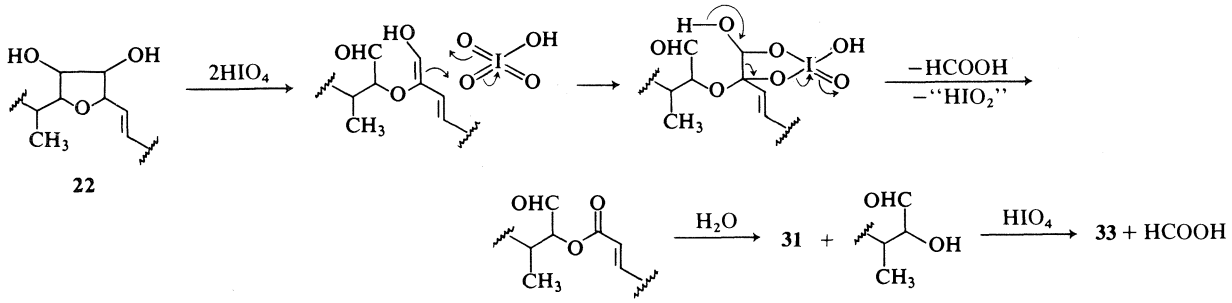
To determine the stereochemistry of the tetrasubstituted tetrahydrofuran ring in goldinamine, the relative configuration was investigated initially (45). Assuming a *cis* relationship between the two vicinal hydroxyl groups, as suggested by the ease of *O*-isopropylidenation and cyclic borate-ester formation, there are only four possible arrangements as depicted in Fig. 10 (**51–54**).

In view of the great variability of the dihedral angles in five-membered rings, and hence of the corresponding coupling constants (46–48), the problem of stereochemical assignments was thought to be resolvable by comparison of coupling constants after increasing conformational rigidity of goldinamine by introduction of a 1,3-dioxolane ring and by selecting model tetrahydrofurans with substituents closely approximating those of *O*-isopropylidene-*N*-(2,4-dinitrophenyl)goldinamine(4-bromobenzyl) ether (**44**). Thus, four model substances **55–58** (Fig. 10) were synthesized by standard reactions from D-mannose (**55** and **57**) and D-glucose (**56** and **58**). Coupling constants for vicinal hydrogens ranged from 3–6 Hz for *cis* and were less than 1 Hz for *trans* relationships. The observed values of 3.5, 6, and 4 Hz in **44** were in agreement with the corresponding coupling constants 3, 6, and 4 Hz in **55** and **57** (Figs. 9 and 10), consistent with an all-*cis* configuration of the tetrahydrofuran moiety in **44** and hence in goldinamine (45).

Chirality of the Tetrahydrofuran Moiety: Absolute Configuration of Aurodox

We had established the geometry of all double bonds, the absolute configuration of the five chiral centers of the goldinonic acid 3,7-hemiacetal portion, the two in the exocyclic part of goldinamine, and the relative configuration of the central tetrahydrofuran ring in goldinamine. There were, therefore, only two structural possibilities for aurodox remaining, differing with respect to enantiomeric tetrahydrofuran rings, but identical otherwise.

A distinction was thought possible by fusing a 6-membered ring onto the 4′–5′ face of the tetrahydrofuran ring in **22** (Scheme 6) containing the two known chiral centers C1 and C2 derived from the exocyclic diene-chain of goldinamine. This approach would lead to a conformationally fixed carbon–carbon bond between two chiral centers. De-



SCHEME 5

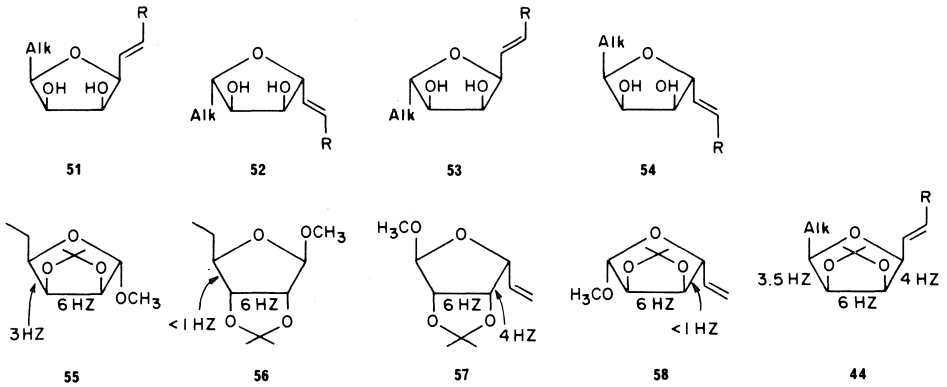


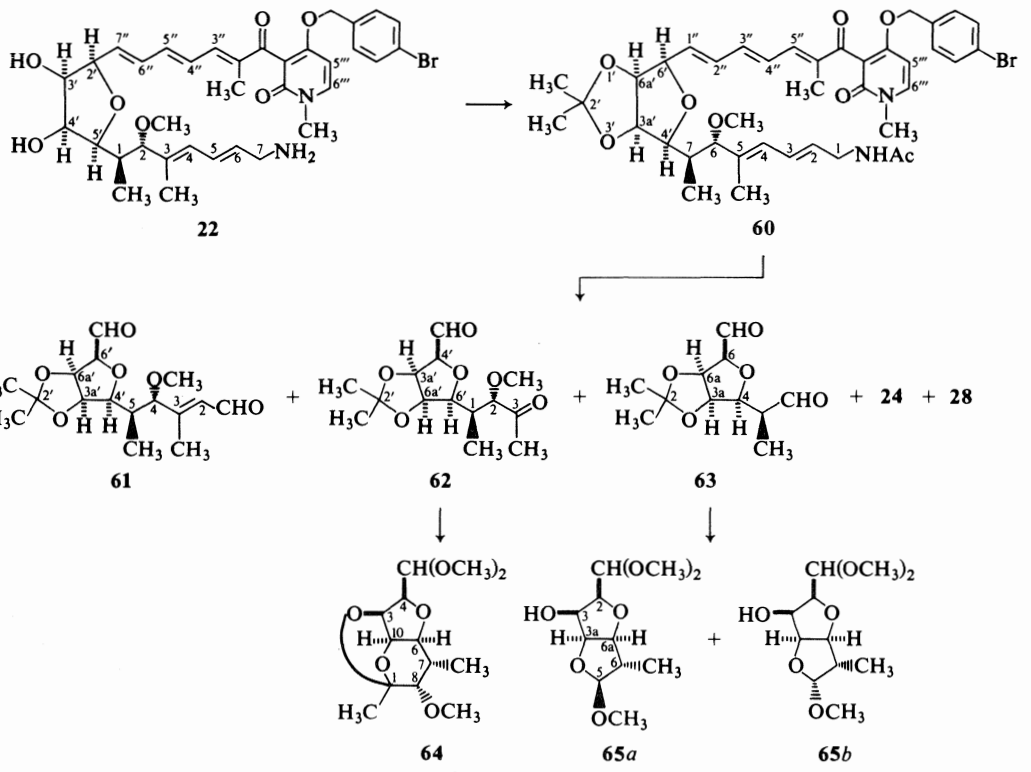
FIG. 10. Possible relative configurations of the tetrahydrofuran moiety in goldinamine (**51–54**) and selected model compounds (**55–58**).

rived from the diene side-chain of goldinamine and designated C1, one of the two carbons possesses an established (*S*) configuration, the other being part of the tetrahydrofuran system. With the known relative configuration of the tetrahydrofuran ring, therefore, determination of the *threo*- or *erythro*-configuration of this bond, on the basis of its coupling constant, would obviously yield the chirality of C5′ in goldinamine and hence the absolute configuration of the entire tetrahydrofuran system. Thus, the *N*-acetyl derivative of **22** was converted to the *O*-isopropylidene compound **60** (Scheme 6)[3] and then oxidatively cleaved with osmium tetroxide and periodate yielding a mixture containing predominantly the α,β-unsaturated aldehyde **61**, smaller quantities of ketone **62** and other minor products, one of which presumably was the dialdehyde **63** as suggested by evidence presented later. The expected pyridones **24** and **28** were also isolated.

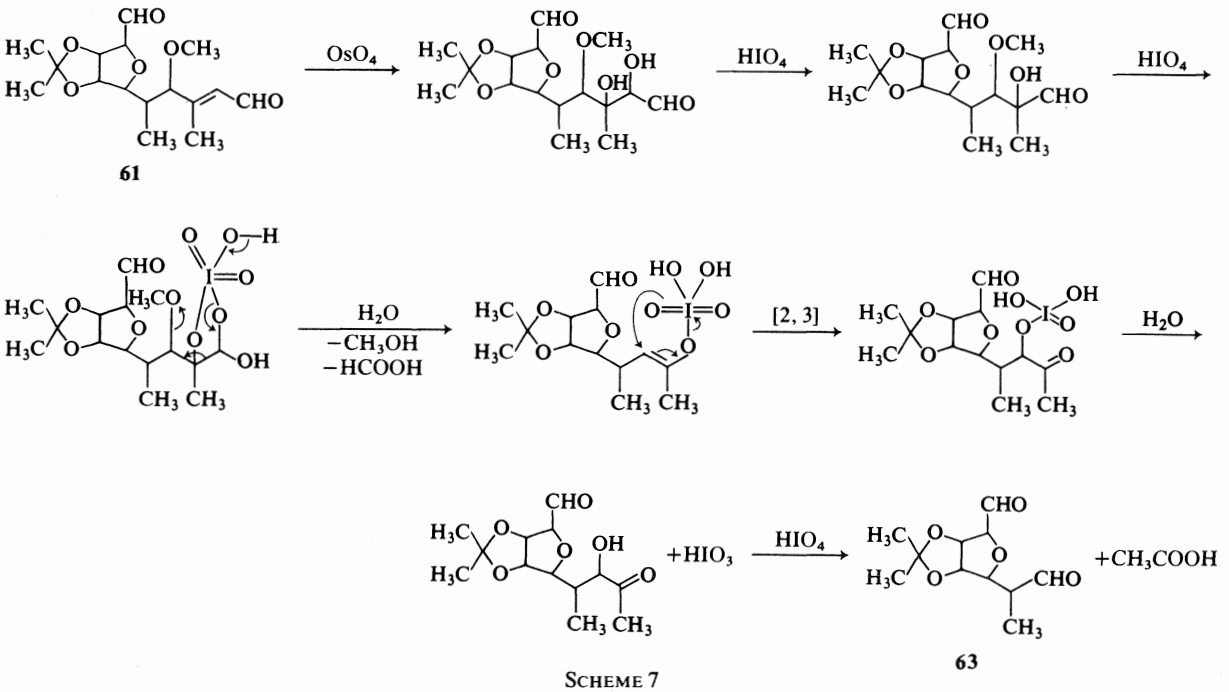
The most attractive starting material for the planned ring closure was the ketone **62**. Consequently, the oxidation mixture derived from **60** was chromatographed on a column of silica gel removing crystalline **24** and **28** and affording a fraction containing **61** as major and **62** as minor products but presumably still contaminated with small amounts of **63**. This mixture was treated with ozone, affording ketone **62** as major product, and subsequently with methanolic hydrogen chloride to effect acetalization of the aldehyde and transacetalization involving the 3-oxo position. This procedure led to a mixture of three products (Scheme 6). The major one was the expected (7*S*,8*S*) - 4 - (dimethoxymethyl) - 1,7 - dimethyl - 8-methoxy - 2,5,9 - trioxatricyclo [4.2.2.$0^{3,10}$] decane (**64**) derived from **62**, whereas the two minor components were identified as the two anomeric (6*S*)-hexahydro - 5 - methoxy - 2 - (dimethoxymethyl) - 6 - methylfuro[3,2-*b*]furan-3-ols (**65***a* and **65***b*) derived from **63**.

Both **62** and **63** contain a diastereotopic carbonyl group in their side chain and a protected chiral diol on their tetrahydrofuran rings. Involving these two groups, transacetalization of **62** occurs in stereorestricted fashion forming stereospecifically but one acetal **64**. Only one of the two potential hydroxyl groups in **63** participates in the transacetalization

[3]To differentiate graphically between relative and absolute configurations we chose bold (▼) and dashed (▽) wedges to indicate absolute stereochemistry, sets of bold lines (▮) and/or broken lines (≡) represent relative configuration.



SCHEME 6

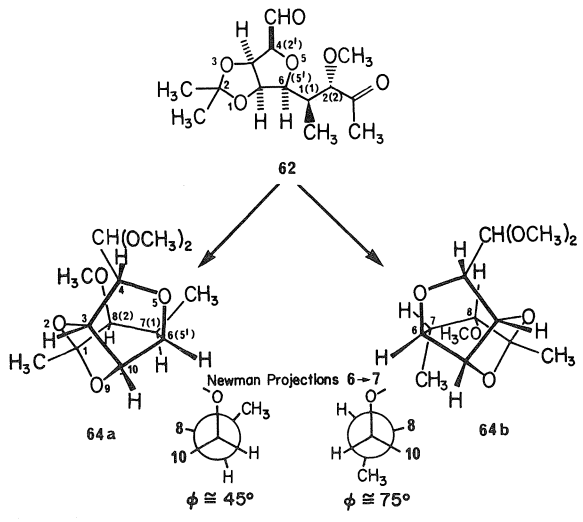


SCHEME 7

process; the second hydroxyl group is donated by the solvent affording two anomeric products **65***a* and **65***b*.

The generation of 2-[2,2-dimethyl-6-formyltetrahydrofuro[3,4-*d*]-1,3-dioxol-4-yl]-2-methylethanal (**63**) from **60** is entirely unexpected but could conceivably be explained as depicted in Scheme 7. Hydroxylation of dialdehyde **61** is followed by glycol cleavage to form a new dialdehyde which forms a cyclic periodic ester. This ester could either decompose to furnish oxoaldehyde **62**, which is actually observed, or the decomposition of the ester could occur with fragmentation as indicated. The resulting mono-coordinated periodic ester could then undergo a 2,3-sigmatropic rearrangement actually reoxygenating position 2 of the side chain and hence yielding an iodic ester which is hydrolyzable to a species cleavable by periodate to yield dialdehyde **63**.

Of the condensation products **64**, **65***a*, and **65***b* the tricyclic acetal **64** appeared most suitable for the ascertainment of chirality. Its existence confirmed the vicinal *cis*-orientation of 3′, 4′, and 5′ in goldinamine and since acetalization generated only one new chiral center in **64** there were only two diastereomeric alternatives **64***a* and **64***b* for consideration (Scheme 8). They are enantiomeric with respect to the acetal carbon 1, derived from the 3-oxo position of **62** and C3 of goldinamine, and the tetrahydrofuran ring, but are identical with respect to the chiral centers 7 and 8 derived from C1 and C2 of **62** and goldinamine. The bond C6—C7 in **64***a* is, therefore, of *threo*- and that in **64***b* of *erythro*-configuration with dihedral angles of ca. 45° and 75°,



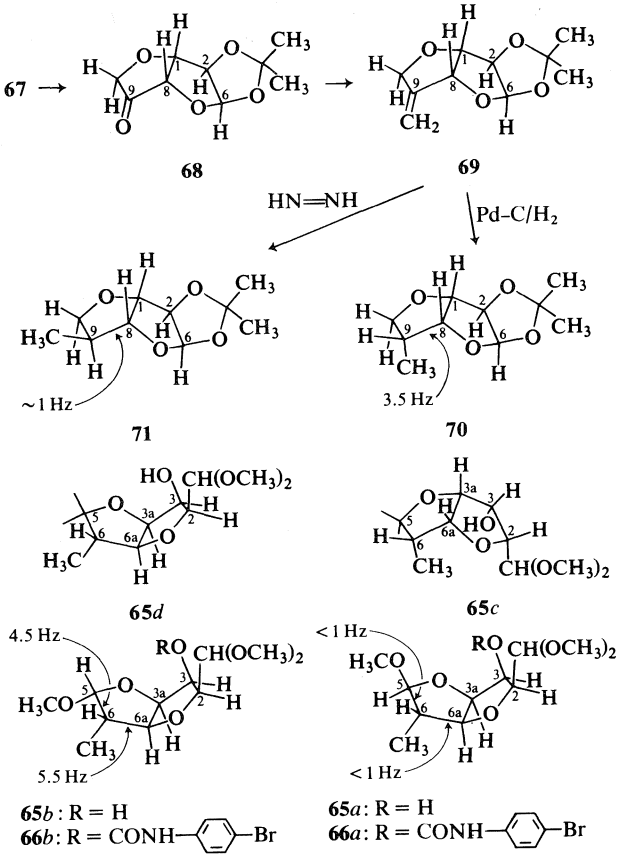
SCHEME 8. Possible configurations of **64**. The numbers shown in parentheses refer to carbon designations of the original goldinamine derivative **60**.

respectively (Scheme 8). Using a $J^{\circ} = 12$ Hz the Karplus equation led to a prediction of $J_{6,7} = 6$ Hz for the *threo* configuration (**64***a*) and $J_{6,7} = 0.5$ Hz for the *erythro* configuration (**64***b*). In view of the observed coupling constant $J_{6,7} = 2$ Hz the *erythro* configuration and hence structure **64***b* appeared somewhat more likely than **64***a*, but no unequivocal assignment was possible by this analysis (49).

The search for an alternative to determine the chirality of C5′ in goldinamine by analysis of the C1—C5′ bond led to the study of the minor methanolysis products **65***a* and **65***b* derived from **60** (Scheme 7). Mass spectrometry established **65***a* and **65***b* as closely related isomers with elemental compositions of $C_{11}H_{20}O_6$, ir spectra suggested the absence of carbonyl groups, and ^1H nmr spectra were compatible with (6*S*)-hexahydro-5-methoxy-2-(dimethoxymethyl)-6-methylfuro[3,2-*b*]furan-3-ols obviously derived from dialdehyde **63**. Concerning their configuration, the chirality of C6 had previously been established by single-crystal analysis of **42** and the relative configuration of the tetrahydrofuran ring, derived from goldinamine, was considered to possess vicinal all-*cis* substituents. Disregarding the configuration at C5, only the two structures **65***c* and **65***d* (Scheme 9) had to be assessed; both are identical with respect to the chirality at C6 but are related enantiomerically with respect to the tetrahydrofuran rings located on the right-hand side. The crucial problem consisted of the differentiation of the two possible bonds between carbons 6 and 6a; a *cis* configuration would establish structure **65***c* and a *trans* relationship structure **65***d*.

To aid analysis, 3,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose (**67**) (58) was oxidized with osmium tetroxide – periodate to [1*R*-(1β,2α,6α,8β]-4,4-dimethyl-3,5,7,11-tetraoxatricyclo[6.3.0.$0^{2,6}$]undecan-9-one (**68**) which served as substrate in a Wittig reaction with triphenylmethylphosphorane leading to olefin **69**. Catalytic hydrogenation of **69** gave the expected [1*S*-(1β,2α,6α,8β,9α)]-4,4,9-trimethyl-3,5,7,11-tetraoxatricyclo[6.3.0.$0^{2,6}$]undecane (**70**) as major (88%) and the 9β isomer **71** as minor product (12%) by preferential hydrogen addition to the double bond from the least hindered side.

Stereoselective hydrogenation with diimide involving hydrogen addition from the more hindered side has been demonstrated, *inter alia*, with 7-oxynorbornadienes via *exo–cis* addition (51). To prepare the 9β-epimer **71** stereoselective hydrogen addition from the more hindered side was required. Accordingly, hydrogenation of olefin **69** was attempted with diimide. This reduction indeed pro-



SCHEME 9

ceeded stereoselectively yielding **71** as major (64%) and **70** (36%) as minor product.

The two model compounds **70** and **71** thus obtained possess identical central tetrahydrofuran rings but differ in the chirality of C9. As a result, **70** exhibits a *cis* and **71** a *trans* configuration between carbons 8 and 9. $J_{8,9} = 1$ Hz allowed unequivocal assignment of structure **71** to the major diimide hydrogenation product; the isomer with $J_{8,9} = 3.5$ Hz, therefore, had to be represented by structure **70**.

The availability of **70** and **71** was thought to enable identification of the methanolysis products derived from **63**, both possessing the same basic moiety of either structure **65***c* or **65***d*. The configurations at centers 8 and 9 of the tetrahydrofuran rings in **70** and **71** resemble the C6—C6a face of **65***c* and **65***d*, respectively, so that $J_{6,6a} \leqq 1$ Hz would point to structure **65***d* and $J_{6,6a} \gg 1$ Hz would favor **65***c*.

Spectral analysis of the two methanolysis products revealed that one of them had two coupling constants $J_{5,6}$ and $J_{6,6a}$ of < 1 Hz each, signifying two vicinal *trans* relationships and hence establishing **65***a* as shown in Scheme 9. The other isomer was most likely the 5-epimer demanding configuration **65***b*.

In view of the close relationship between the configurations represented by **65***a* and **65***b*, the two greatly different coupling constants $J_{6,6a}$ of < 1 Hz (**65***a*) and 5.5 Hz (**65***b*) did not appear to support a *trans* configuration between carbons 6 and 6a in structure **65***b*, a conclusion supported by the *trans* coupling constant $J_{8,9} = 1$ Hz in **70**. Hence, doubt was cast upon the chiral integrity of C6. Racemization at C6 was unlikely, however, in view of the reaction conditions employed and because only two methanolysis products derived from **63** were observed, both of which exhibited virtually identical chemical shifts of the 6-CH_3 groups but differed markedly in the chemical shifts of H5. With two oxygen substituents on the same ring side, moreover, H5 of **65***b* would be expected to absorb at lower field than H5 of **65***a*, and it is indeed observed that H5 of **65***b* absorbs at a field 0.29 ppm lower than H5 of **65***a*. C5 in **65***b* was thus certainly of the *R*-configuration shown. One of the two coupling constants $J_{5,6} = 4.5$ and $J_{6,6a} = 5.5$ Hz in **65***b* therefore had to be assigned to a *trans* configuration since one vicinal *cis* and one *trans* configuration between carbons 5, 6, and 6a must be involved regardless of the chirality of C6.

These results could be summarized as follows. The *R*-configuration of **63** is most probably preserved in the methanolysis products **65***a* and **65***b*. Structure **65***a* (Scheme 9) could then be assigned to one of the methanolysis products in view of $J_{5,6} = J_{6,6a} < 1$ Hz. The C6—C6a bond in **65***a* and **65***b*, and the C1—C5′ bond in goldinamine are of *erythro* configuration so that **64***b* is indeed the structure of the methanolysis product of **63** and C5′ of goldinamine has the *S*-configuration.

A single-crystal Roentgen analysis of **64** subsequently established **64***b* as the correct structure (49). It was also possible to prepare and analyze the crystalline hexahydro-5-methoxy-2-(dimethoxymethyl)-6-methylfuro[3,2-*b*]furan-3-ol(4-bromophenyl)carbamic acid esters **66***a* and **66***b* (Fig. 11) confirming the structures **65***a* and **65***b* as the methanolysis products derived from **63**.

The crystal structure analyses of **64***b* and **66***b* yielded the absolute configurations which, in turn, were in agreement with the previously determined chiral centers of **42**. Thus, the previously assigned all-*cis* configuration of the tetra-substituted tetrahydrofuran ring in goldinamine, the *erythro* configurations of C6—C6a, the chiral integrities of C6, and the epimeric relationship in **65***a* and **65***b* were confirmed.

These studies and others (52) demonstrated that

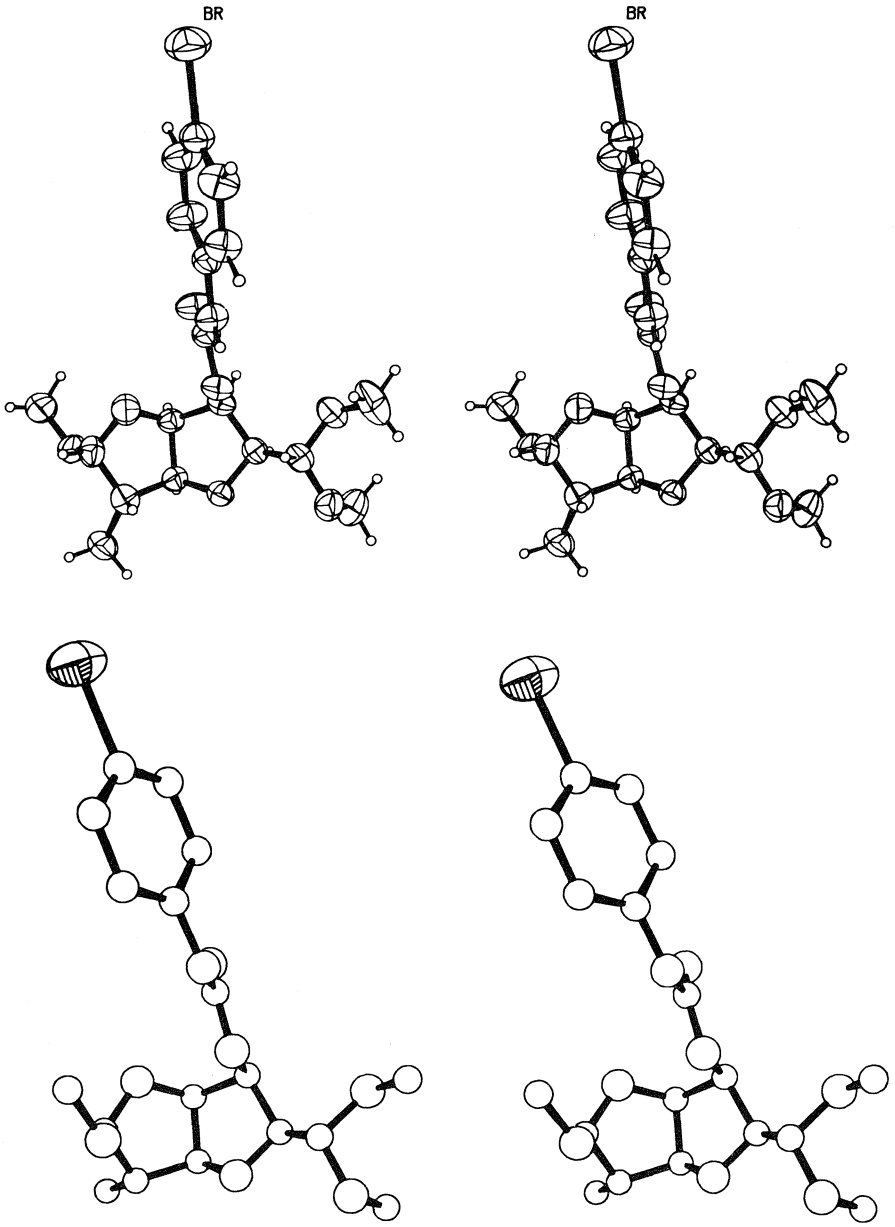


FIG. 11. Stereodrawings showing the conformations and absolute configurations of **66***b* (upper) and **66***a* (lower). The thermal ellipsoids of the anisotropic atoms are scaled to 50% probability level. The isotropic atoms are shown as spheres of arbitrary size.

vicinal coupling constants in the relatively rigid tetrahydrofuro[3,4-*d*]-1,3-dioxoles and tetrahydrofuro[2,3-*d*]-1,3-dioxoles can vary over a range of 0–6.5 Hz yielding very little configurational information. The only permissible conclusion appears to be based on $J \leqq 1$ Hz diagnostic for *trans* configurations. We seemed to have demonstrated convincingly the relative configuration of the tetrahydrofuran ring in goldinamine by ^{1}H nmr spectroscopy in conjunction with model compounds **55–58** and the resulting conclusions turned out to be correct. The study of the 5-epimers **65***a* and **65***b* as well as the 9-epimers **70** and **71** explicitly showed, however, that any extension of assignments due to coupling constants considerably larger than 1 Hz, although based on carefully chosen model systems, will remain problematic.

With the completed structure analyses of **64**, **65***a*,

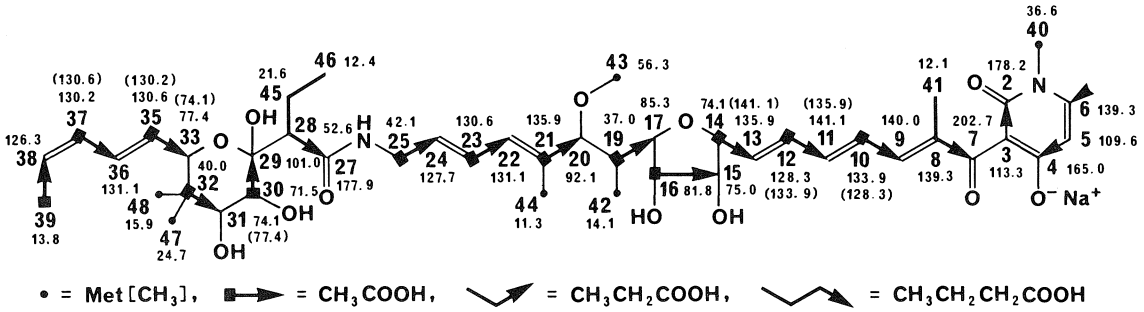


FIG. 12. Biosynthetic pattern and ^{13}C nmr chemical shifts of aurodox sodium salt.

and **65***b* the last stereochemical problems were resolved so that the structure of aurodox can be represented by **1***b*.

Configuration and Chirality of Mocimycin

Constitutional parity of des-*N*-methyl aurodox (**1***a*) with mocimycin (53, 54) was likely in view of very similar nmr spectra of aurodox (**1***b*) and mocimycin, differing only by the presence or absence, respectively, of an *N*-methyl signal. Proof for this assumption was furnished (8) by the demonstration that mocimycin gave mono- and dimethylated products identified as des-*N*-methyl **17** and **17**, the latter also prepared from **1***b* (Scheme 3). Both methylation products of **1***a*, upon treatment with acetic acid, yielded goldinono-1,4-lactone-3,7-hemiacetal (**13**) identical with authentic **13** as shown by spectral and chiroptical properties, and also afforded des-*N*-methyl **21** and **21**, respectively. The latter two goldinamine derivatives, both derived from mocimycin, were *N*-trifluoroacetylated furnishing **46** and **47**, respectively, whose elemental formulas were deduced by mass spectrometry. Further, **47** was also prepared from aurodox via **21** and exhibited a mass spectrometric fragmentation pattern identical to **47** from mocimycin (8). Configurational and chiral parity of the goldinamine moieties of mocimycin and goldinamine was conclusively demonstrated (8) by identical ord and cd spectra of **47** derived from **1***a* and **1***b*, by additional degradation and derivatization reactions of mocimycin yielding des-*N*-methyl-**29**, **29**, [4*E*, 6*E*, 2*R*, 3*S*]-8-acetylamino-3-methoxy-2,4-dimethyl-4,6-octadienal (**34**), and (1*R*,3*R*,4*S*,6*S*,7*S*,8*S*,10*R*)-4-(dimethoxymethyl)-1,7-dimethyl-8-methoxy-2,5,9-trioxatricyclo [4.2.2.$0^{3,10}$]decane (**64***b*), encompassing all remaining chiral centers.

Biosynthesis and ^{13}C nmr Spectrum of Aurodox

The availability of various degradation products of aurodox permitted the ^{13}C spectral assignments of aurodox with very few ambiguities remaining.[4] As expected, the 14 olefin methine carbons, especially C9–C13 of the trienone portion, posed problems in view of their similar chemical shifts. The incorporation of acetate, previously demonstrated (55, 56), was used to divide C9–C13 into two categories: C9, C11, and C13 derived from [1-^{13}C]-acetate, and C10 and C12 from [2-^{13}C]acetate. Assigning C9 by SFOR residual coupling led to the spectral results summarized in Fig. 12.

Biosynthetic studies with ^{14}C- and ^{13}C-labeled precursors (55, 56) showed the goldinonic acid portion derived from five acetate and one butyrate units with the geminal dimethyl group at C32 labeled by L-[Me-^{13}C]methionine. The goldinamine portion is derived from eight consecutive acetate units involving a sixteen-carbon polyketide chain methylated at C19 and C21, and a propionate unit. The origin of the pyridone moiety is somewhat obscure, in part due to general peak-broadness of C3 and C5 in [2-^{13}C]acetate labeled aurodox. The peak at 139.3, now assigned to C6, showed threefold enrichment in [1-^{13}C]acetate labeled aurodox while C2 and C4 were two and sevenfold enriched, respectively. It appears, therefore, that acetate is involved in the biosynthesis of the pyridone ring although a detailed proposal remains to be determined. The biosynthetic results are summarized in Fig. 12.

Experimental

All evaporations were carried out at reduced pressure. For chromatography we used silica gel (Woelm, <0.08 mm), silicic acid (Mallinckrodt, 100 mesh), and precoated tlc plates (Merck, silica gel F-254 for analytical and Merck, silica gel PF-254 for preparative plates). All solvent ratios are v/v. The following solvent systems were employed: chloroform–methanol – concentrated ammonium hydroxide, 40:10:1 (*A*); chloroform – ethyl acetate – methanol, 5:5:1, (*B*); chloroform – diethyl ether, 1:1 (*C*); chloroform – diethyl ether, 1:2 (*D*); chloroform – diethyl ether, 2:1 (*E*); chloroform–methanol, 4:1 (*F*); chloroform–methanol, 9:1 (*G*); cyclohexane – diethyl ether, 1:1 (*H*); cyclohexane–chloroform, 1:1 (*I*); and 1-butanol – acetic acid – water, 4:1:1 (*J*). Spots were visualized under uv light unless stated otherwise. Melting points were obtained on a hot stage (Reichert, Thermopan) and are uncorrected. ^1H and ^{13}C nmr spectra were obtained on a Varian XL-100 spectrometer in the

[4]Unpublished results.

continuous-wave mode and Fourier-transform mode, respectively.

(3S, 4E, 6Z)-3-Hydroxy-2,2-dimethyl-4,6-octadienal (2)

Aurodox sodium salt (4.2 g, 5 mmol) was dissolved in water (200 mL) containing sodium hydrogen carbonate (4 g). The solution was diluted with dioxane (400 mL), cooled in an ice bath, and further diluted with an ice-cold solution of sodium metaperiodate (10.7 g, 50 mmol) in water (400 mL). The reaction mixture was stirred at ca. 4°C for 20 h, immersed in an ice bath, quenched by the addition of ethylene glycol (5.6 mL), stirred for 2 h, and extracted with diethyl ether (4 × 400 mL). The combined extracts were washed with water (2 × 150 mL) and dried. Evaporation of the solvent gave crude **2** as oil (830 mg), R_f 0.60 (*B*). A small sample was purified by preparative tlc; uv (dioxane) λ_{max}: 235(ε 18770, diene) nm; *Mol. Wt.* calcd. for $C_{10}H_{16}O_2$: 168.24; found (ms) *m/e* (%): 168 (38), 97 (100).

(3S, 4E, 6Z)-2,2-Dimethyl-4,6-octadiene-1,3-diol (3)

A solution of crude **2** (820 mg) in diethyl ether (75 mL) was added dropwise over a 30 min period to a stirred mixture of lithium aluminum hydride (2 g) in diethyl ether (300 mL). After stirring for 4 h at 5°C excess hydride was destroyed by dropwise addition of saturated sodium sulfate solution. The ether layer was removed by decantation, the white precipitate was washed with ether, and the ethereal solutions were combined, dried, and concentrated to a syrup of crude **3** (810 mg). A solution of this material in chloroform was chromatographed on a column of silica gel (25 g) with chloroform (200 mL) and system *E* as mobile phase affording pure **3** as a syrup, R_f 0.55 (*B*), 0.28 (*E*); uv (2-propanol) λ_{max}: 233 (ε 24700) nm. The ^{1}H nmr spectrum is shown in Fig. 1. *Mol. Wt.* calcd. for $C_{10}H_{18}O_2$: 170.25; found (ms) *m/e* (%): 170 (6), 97 (100).

Aurodox (4-Bromobenzyl) Ether (18)

4-Bromobenzyl bromide (3 g, 12 mmol) was added to a solution of **1***b* sodium salt (8.33 g, 10 mmol) in dimethylformamide (125 mL). The solution was kept for 3 days at room temperature in the dark, transferred to a separatory funnel with ethyl acetate (1 L) and saturated sodium hydrogen carbonate solution (350 mL). The organic phase was washed once with sodium hydrogen carbonate solution (350 mL), three times with water (100 mL each), and dried ($MgSO_4$). Concentration gave a thin, yellow syrup which was dissolved in acetone (20 mL) and chromatographed on a column of Sephadex LH-20 (9 cm × 47 cm) with acetone as mobile phase. Fractions containing pure **18** were combined (tlc), concentrated to a volume of 50 mL, and slowly added to ether (500 mL) under vigorous stirring. After further addition of petroleum ether (500 mL) the precipitate was collected by filtration, washed with ether and petroleum ether, and dried under reduced pressure, R_f 0.72 (*A*), 0.20 (*B*). *Anal.* calcd. for $C_{51}H_{67}BrN_2O_{12}\cdot H_2O$ (998.03): C 61.38, H 6.97, N 2.81, Br 8.01; found: C 61.37, H 7.14, N 2.71, Br 8.18.

*[1R-(1β,3α,5α,6β,9α,E,Z)]-9-Ethyl-1,5-dihydroxy-4,4-dimethyl-3-(1,3-pentadienyl)-2,7-dioxabicyclo-[4.3.0]nonan-8-one (Goldinono-1,4-lactone-3,7-hemiacetal, **13**) and [2R,3R,4S,5S,1(R*),2(S*)]-4-(4-Bromobenzyloxy)-1-methyl-3-[7-[5-[7-amino-2-methoxy-1,3-dimethyl-3(E),5(E)-heptadienyl]-3,4-dihydroxytetrahydro-2-furyl]-2-methyl-1-oxo-2(E),-4(E),6(E)-heptatrienyl]-2(1H)-pyridinone (Goldinamine (4-Bromobenzyl) Ether, **22**)*

A solution of **18** (400 mg) in acetic acid (10 mL) was kept at room temperature for three days in the dark or was heated on the steam bath for 20 min. The acetic acid was removed by distillation and codistillation with toluene. The residue was dissolved in chloroform and chromatographed on a column containing silicic acid (35 g). Elution started with chloroform (fractions 1–6, 12 mL each) and was followed by chloroform–methanol, 20:1 (7–20), chloroform–methanol, 4:1 (21–40), and chloroform–methanol – acetic acid, 16:4:1 (41–86). Fractions 14–17 contained crude **13** (111 mg, R_f 0.24, (*A*)) which were purified by rechromatography or preparative tlc (0.48 (*B*)) to give white crystals of **13**, mp 110°C; $[\alpha]_D$ −23° (*c* 1.0, $CHCl_3$) (37). *Anal.* calcd. for $C_{16}H_{24}O_5$ (296.35): C 64.84, H 8.16; found: C 64.56, H 8.29. Fractions 54–77 were pooled and concentrated to a syrup, redissolved in water and freeze-dried to yield amorphous **22** acetic acid salt hemihydrate (116 mg), R_f 0.6 (*A*); nmr (CD_3OD)[5] δ: 0.82 (d, 3, $J_{1,Me}$ = 7 Hz, 1-Me), 1.71 (s, 3, 3-Me), 1.89 (s, 3, $MeCOO^-$), 1.94 (s, 3, 2″Me), 2.20 (m, 1, $J_{1,Me}$ = 7 Hz, H1), 3.15 (s, 3, OMe), 3.37 (d, 1, $J_{1,2}$ = 10 Hz, H2), 3.47 (s, 3, NMe), 3.57 (d, 2, $J_{6,7}$ = 6 Hz, H7), 3.71 (dd, 1, $J_{1,5'}$ = 7 and $J_{4',5'}$ = 4 Hz, H5′), 4.19 (dd, 1, $J_{3',4'}$ = 5 and $J_{4',5'}$ = 4 Hz, H4′), 4.32 (m, 2, H2′ and H3′), 5.11 (s, 2, CH_2—C_6H_5), 5.75 (dt, 1, $J_{5,6}$ = 15 and $J_{6,7}$ = 7 Hz, H6), 6.03 (d, 1, $J_{4,5}$ = 10 Hz, H4), 6.08 (dd, 1, $J_{2',7''}$ = 7 and $J_{6'',7''}$ = 14 Hz, H7″), 6.41 (dd, 1, $J_{5'',6''}$ = 11 and $J_{6'',7''}$ = 14 Hz, H6″), 6.43 (d, 1, $J_{5''',6'''}$ = 7.5 Hz, H5‴), 6.48 (dd, 1, $J_{4''5''}$ = 14 and $J_{5''6''}$ = 11 Hz, H5″), 6.61 (dd, 1, $J_{4,5}$ = 10 and $J_{5,6}$ = 15 Hz, H5), 6.68 (dd, 1, $J_{3''4''}$ = 11 and $J_{4'',5''}$ = 14 Hz, H4″), 6.91 (d, $J_{3'',4''}$ = 11 Hz, H3″), 7.18, 7.43 (AA′BB′, 4, J_0 = 8.5 Hz, C_6H_4), and 7.70 (d, 1, $J_{5''',6'''}$ = 7.5 Hz, H6‴). *Anal.* calcd. for $C_{35}H_{43}BrN_2O_7\cdot CH_3COOH\cdot\frac{1}{2}H_2O$ (752.71): C 59.04, H 6.43, N 3.72, Br 10.62; found: C 59.01, H 6.33, N 3.77, Br 10.89.

The free base of **22** was prepared by dissolving **22** acetic acid salt (50 g) in water (5 mL) and adding 0.1 *N* sodium hydroxide solution to pH 9.2. The precipitate was collected, washed with water, and dried to yield a light tan, amorphous powder; nmr ($CDCl_3$) similar to **22** acetic acid salt but lacking a signal for acetate, and H7 shifted diamagnetically to δ 3.28.

[2S,3S,4R,5R,7(R),6(S*)]-N-[7-[5-[7-[(4-Bromobenzyloxy)-1,2-dihydro-1-methyl-2-oxo-3-pyridinyl]-6-methyl-7-oxo-1(E),3(E),5(E)-heptatrienyl]tetrahydro-3,4-dihydroxy-furan-2-yl]-6-methoxy-5,7-dimethyl-2(E),4(E)-heptadienyl]acetamide (N-Acetyl **22**)*

Acetic anhydride (8 mL) was added to a solution of **22** acetic acid salt (2 g) in methanol (20 mL). After one hour the mixture was added dropwise to a rapidly stirred mixture of ether (700 mL) and petroleum ether (600 mL). The precipitate was collected, dried, redissolved in acetone (4 mL), and chromatographed on a column of Sephadex LH-20 (51 mm × 510 mm) with acetone as mobile phase. Pure fractions were pooled, evaporated, and redissolved in acetone. The resulting solution was added dropwise to rapidly stirred petroleum ether (400 mL). The precipitate was collected by filtration and dried to give an amorphous powder (1.65 g) (R_f 0.15 (*B*); 0.85 (*F*); 0.50 (*G*)); nmr (CD_3OD) similar to that of **22** acetic acid salt, but shows δ 1.94 (s, 3, N—Ac).

[2S,3R,4S,5R,7(R),6(S*)]-N-[7-[5-[7-[(4-Bromobenzyloxy)-1,2-dihydro-1-methyl-2-oxo-3-pyridinyl]-6-methyl-7-oxo-1(E),3(E),5(E)-heptatrienyl]tetrahydro-3,4-bis(acetyloxy)-furan-2-yl]-6-methoxy-5,7-dimethyl-2(E),4(E)-heptadienyl]acetamide (N,O,O-Triacetyl **22**)*

A solution of **22** acetic acid salt (150 mg) was dissolved in anhydrous pyridine (4 mL) and acetic anhydride (2 mL) was added. The solution was kept in the dark overnight, evaporated to dryness, the residue dissolved in chloroform, and chromatographed on silica gel (8 g). With system *B* as mobile phase pure fractions were obtained which were pooled and evaporated. Dissolving the residue in chloroform and precipitating with petroleum ether gave amorphous *N,O,O*-triacetyl **22** (140 mg), R_f 0.25 (*B*); nmr (CD_3OD) δ: 0.85 (d, 3, $J_{7,Me}$ = 7 Hz, 7-Me), 1.63 (s, 3, 5-Me), 1.92, 1.95, 2.04 (3s, 9, CH_3CO), 1.98 (s, 3, 6″-Me), 2.21 (m, 1, H7), 3.03 (s, 3, CH_3O), 3.23 (d, 1, $J_{6,7}$ = 10 Hz, H6), 3.50 (s, 3, NMe), 3.86 (d, 2, $J_{1,2}$ = 6 Hz, H1), 3.92 (dd, 1, $J_{2',7'}$ = 7 and

[5]For proton designations see Scheme 6.

$J_{2',3'}$ = 3 Hz, H2'), 4.57 (dd, 1, $J_{4',5'}$ = 6 and $J_{1'',5'}$ = 7 Hz, H5'), 5.11 (s, 2, CH_2—C_6H_5), 5.41 (dd, 1, $J_{4',5'}$ = 6, $J_{3',4'}$ = 4.5 Hz, H4'), 5.46 (t, 1, $J_{3',4'} = J_{2',3'}$ = 4.5 Hz, H3'), 5.64 (dt, 1, $J_{2,3}$ = 15 and $J_{1,2}$ = 6 Hz, H2), 5.84 (dd, 1, $J_{1'',5'}$ = 7 and $J_{1'',2''}$ = 14.5 Hz, H1''), 5.94 (d, 1, $J_{4,5}$ = 11 Hz, H4), 6.38 (dd, 1, $J_{2'',3''}$ = 11 and $J_{1'',2''}$ = 14 Hz, H2''), 6.41 (d, 1, $J_{5''',6'''}$ = 7.5 Hz, H5'''), 6.45 (dd, 1, $J_{3'',4''}$ = 14 and $J_{2'',3''}$ = 11 Hz, H3''), 6.50 (dd, 1, $J_{4,5}$ = 11 and $J_{5,6}$ = 15 Hz, H3), 6.69 (dd, 1, $J_{4'',5''}$ = 11 and $J_{3'',4''}$ = 14 Hz, H4''), 6.87 (d, 1, $J_{4'',5''}$ = 11 Hz, H5''), 7.17, 7.41 (AA'BB', 4, J_0 = 8.5 Hz, C_6H_5), and 7.67 (d, 1, $J_{5''',6'''}$ = 7.5 Hz, H6''').

[2R,3R,4S,5S,1(R),2(S*)]-4-(4-Bromobenzyloxy)-1-methyl-3-[7-[5-[7-(2,4-dinitrophenylamino)-1,3-dimethyl-2-methoxy-3(E),5(E)-heptadienyl]-3,4-dihydroxytetrahydro-2-furyl]-2-methyl-1-oxo-2(E),4(E),6(E)-heptatrienyl]-2(1H)-pyridone (**26**)*

2,4-Dinitrofluorobenzene (0.35 mL) was added to a mixture of **22** acetic acid salt (0.5 g), potassium carbonate (1 g), and acetone (30 mL). After shaking for 20 h in the dark, the suspension was filtered, the filtrate evaporated, and the residue chromatographed on a column of Sephadex LH-20 (25 mm × 510 mm) with acetone as mobile phase to furnish **26** by precipitation with petroleum ether from concentrated solution (0.3 g), R_f 0.71 (*G*); nmr ($CDCl_3$)[6] δ: 0.87 (d, 3, $J_{1,Me}$ = 7 Hz, 1-Me), 1.70 (s, 3, 3-Me), 2.02 (s, 3, 2''-Me), 2.20 (m, 1, H1), 3.22 (s, 3, 2-OMe), 3.31 (d, 1, $J_{1,2}$ = 9.5 Hz, H2), 3.50 (s, 3, NMe), 3.56 (m, 1, H5'), 4.22 (m, 3, NCH_2 and H2'), 4.42 (m, 2, H3' and H4'), 5.03 (s, 2, OCH_2), 5.85 (dt, 1, $J_{5,6}$ = 15.5 and $J_{6,7}$ = 6 Hz, H6), 6.06 (d, 1, $J_{5''',6'''}$ = 7.5 Hz, H5'''), 6.06 (m, 2, H4 and H7''), 6.30–6.80 (m, 4, H4'', H5, H5'', H6''), 6.90 (d, 1, $J_{3'',4''}$ = 10 Hz, H3''), 6.99 (d, 1, $J_{5,6}$ = 9 Hz, H6 of DNP), 7.17, 7.48 (AA'BB', 4, J_0 = 8.5 Hz, C_6H_4), 7.35 (d, 1, $J_{5''',6'''}$ = 7.5 Hz, H6'''), 8.35 (dd, 1, $J_{3,5}$ = 3 and $J_{5,6}$ = 9 Hz, H3 of DNP), 8.72 (t, 1, $J_{7,NH}$ = 6 Hz, NH), 9.20 (d, 1, $J_{3,5}$ = 3 Hz, H5 of DNP). A 220 MHz spectrum is shown in Fig. 8.

[2R,3S,4R,5S,1(R),2(S*)]-4-(4-Bromobenzyloxy)-1-methyl-3-[7-[5-[7-(2,4-dinitrophenylamino)-2-methoxy-1,3-dimethyl-3(E),5(E)-heptadienyl]-3,4-bis(acetyloxy)tetrahydrofuran-2-yl]-2-methyl-1-oxo-2-methyl-2(E),4(E),6(E)-heptatrienyl]-2(1H)-pyridinone (**43**)*

A mixture of **26** (0.30 g), pyridine (3 mL), and acetic anhydride (1.5 mL) was stirred at 25°C for 26 h and then added dropwise to diethyl ether – petroleum ether (1:2, 200 mL). The resulting precipitate of crude **43** (0.29 g) was chromatographed on Sephadex LH-20 (acetone) yielding pure **43** (0.26 g), R_f 0.52 (*B*); nmr shown in Fig. 8.

[3aS,4R,6aR,6S,1(R),2(S*)]-4-(4-Bromobenzyloxy)-1-methyl-3-[7-[6-[7-(2,4-dinitrophenylamino)-2-methoxy-1,3-dimethyl-3(E),5(E)-heptadienyl]-tetrahydro-2,2-dimethyl-furo[3,4-d]-1,3-dioxol-4-yl]-2-methyl-1-oxo-2(E),4(E),6(E)-heptatrienyl]-2(1H)-pyridinone (**44**)*

2,2-Dimethoxypropane (0.5 mL) and bis(4-nitrophenyl) phosphate (75 mg) were added to a solution of **26** (100 mg) in acetone (5 mL). The mixture was neutralized with Dowex 1 (HCO_3^-) after 2 h, the resin filtrate and washings (methanol) were evaporated, and the residue chromatographed on Sephadex LH-20 (acetone) to yield pure **44** as yellow powder by precipitation with petroleum ether, R_f 0.52 (*B*); nmr ($CDCl_3$)[7] δ: 0.93 (d, 3, $J_{1,Me}$ = 6 Hz, 1-Me), 1.29, 1.43 (2s, 3 each, 2-Me_2), 1.70 (s, 3, 3-Me), 2.03 (s, 3, 2''-Me), 2.31 (m, 1, H1), 3.18 (s, 3, 2-OMe), 3.36 (d, 1, $J_{1,2}$ = 9.5 Hz, H2), 3.49 (s, 3, NMe), 3.57 (dd, 1, $J_{1',6}$ = 6 and $J_{6',6a'}$ = 3.5 Hz, H6'), 3.97 (dd, 1, $J_{3a',4'}$ = 4 and $J_{4',7''}$ = 8 Hz, H4'), 4.16 (t, 2, $J_{6,7} = J_{7,NH}$ = 6 Hz, NCH_2), 4.62 (dd, 1, $J_{3a',4'}$ = 4 and $J_{3a',6a'}$ = 6 Hz, H3a'), 4.71 (dd, 1, $J_{3a',6a'}$ = 6 Hz and $J_{6',6a'}$ = 3.5 Hz, H6a'), 5.03 (s, 2, OCH_2), 5.75 (dt, 1, $J_{5,6}$ = 15 and $J_{6,7}$ = 6 Hz, H6), 6.02 (d, 1, $J_{5''',6'''}$ = 7.5 Hz, H5'''), 6.03 (m, 2, H4 and H7''), 6.30–6.80 (m, 4, H4'', H5, H5'', H6), 6.88 (d, 1, $J_{3'',4''}$ = 10 Hz, H3''), 6.95 (d, 1, $J_{5,6}$ = 9 Hz, H6 of DNP), 7.13, 7.45 (AA'BB', 4, J_0 = 8.5 Hz, C_6H_4), 7.37 (d, 1, $J_{5''',6'''}$ = 7.5 Hz, H6'''), 8.28 (dd, 1, $J_{3,5}$ = 3 and $J_{5,6}$ = 9 Hz, H5 of DNP), 8.65 (t, 1, $J_{7,NH}$ = 6 Hz, NH), 9.15 (d, 1, $J_{3,5}$ = 3 Hz, H3 of DNP).

*[1R-(1β,3α,5α,6β,9α)]-9-Ethyl-1,5-dihydroxy-4,4-dimethyl-3-pentyl-2,7-dioxabicyclo[4.3.0]nonan-8-one (Tetrahydrogoldinono-1,4-lactone-3,7-hemiacetal, **14**)*

Crude **13** (2 g) in acetic acid (30 mL) was stirred with pre-hydrogenated platinum oxide (160 mg) for 8 h under hydrogen pressure of 2 atm. Removal of the catalyst and solvent gave a syrup which was dissolved in chloroform and chromatographed on alumina (40 g). Changing to chloroform – ethyl acetate (9:1) as the mobile phase eluted **14**. Final purification was achieved by chromatography on Sephadex LH-20 (50 mm × 570 mm) with acetone as mobile phase. Crystallization from ethanol–water gave white needles (1.2 g), R_f 0.60 (*B*) (37).

*4-Hydroxy-1-methyl-2(1H)-pyridone (**19**)*

A solution of **1***b* sodium salt (10 g) in ethanol (150 mL) was hydrogenated (3.5 atm) over 10% palladium-on-barium sulfate (2 g) for 6.5 h. The catalyst was filtered off, the filtrate was diluted with water (150 mL), refluxed for 5 h, and evaporated to dryness. The dry residue was extracted three times with chloroform, the remaining solids were dissolved in methanol–water, 2:1, and the solution passed through a column of Dowex 50 X-4 (200–400 mesh, H^+, 50 mL). The resin was rinsed with methanol–water (2:1, 100 mL) followed by methanol–water–pyridine (2:2:1) which eluted **19**. Pooling and concentration of the appropriate fractions gave a syrup which solidified on standing. Recrystallization from ethanol–diethyl ether gave large prisms of **19** (700 mg), R_f 0.19 (*A*), 0.25 (*B*); mp 168–170°C. *Anal.* calcd. for $C_6H_7NO_2$ (125.14): C 57.59, H 5.64, N 11.19; found: C 57.62, H 5.75, N 11.13; (40).

The acid was converted to the thallium salt with thallium ethoxide and recrystallized from methanol–ethanol–ether to afford colorless needles, mp 203–204°C. *Anal.* calcd. for $C_6H_6NO_2Tl$ (328.51): C 21.94, H 1.84, N 4.26; found: C 21.83, H 1.71, N 4.26.

*Methylenebis(4-hydroxy-1-methyl-2(1H)-pyridinone) (**20**)*

Formaldehyde solution (37%, 0.1 mL) and piperidine (one drop) were added to a solution of **19** (120 mg) in ethanol–water (1:1, 4 mL). The mixture was refluxed on the steam bath for 10 min and refrigerated. The precipitate was recrystallized from ethanol–chloroform (84 mg), R_f 0.80 (*A*); mp 300–305°C; uv λ_{max} (ε): 282 (11 580) in 0.1 *N* HCl, 281–282 (12 900) nm in 0.1 *N* KOH; nmr ($CDCl_3$) δ: 3.49 (s, 6, 2 NMe), 3.74 (s, 2, CH_2), 6.09 (d, 1, $J_{5,6}$ = 8 Hz, H5), 7.06 (d, 1, $J_{5,6}$ = 8 Hz, H6), and 13.01 (s, 2, 2 OH). *Anal.* calcd. for $C_{13}H_{14}N_2O_4$ (262.26): C 59.4, H 5.38, N 10.68; found: C 59.72, H 5.35, N 10.52.

*4-(4-Bromobenzyloxy)-1,2-dihydro-1-methyl-2-oxopyridine-3-carboxylic Acid (**24**)*

A solution of potassium carbonate (4.14 g), sodium metaperiodate (8.5 g), and potassium permanganate (63 mg) in water (400 mL) was added to a solution of **22** acetic acid salt (500 mg) in 90% *tert*-butanol (500 mL). The mixture was stirred overnight at room temperature in the dark, acidified (pH 1.5, 6 *N* sulfuric acid), and extracted with diethyl ether (500 mL). The dried extract was evaporated and the residue triturated repeatedly with chloroform. The remaining solids were then extracted with chloroform–methanol (9:1, 50 mL). This extract was evaporated and the residue allowed to crystallize from hot methanol. Recrystallization from methanol–ethanol–water gave colorless

[6]For proton designations see Fig. 8.

[7]For proton designations see Fig. 9.

prisms of **24**, R_f 0.10 (*A*); mp 224°C. *Anal.* calcd. for $C_{14}H_{12}BrNO_4$ (338.16): C 49.73, H 3.58, N 4.14; found: C 49.68, H 3.58, N 4.23 (40).

*4-(4-Bromobenzyloxy)-1,2-dihydro-1-methyl-2-oxopyridine-3-carboxylic Acid Methyl Ester (**25**)*

Diethyl ether (2 mL) containing an excess of diazomethane was added to a suspension of **24** (15 mg) in tetrahydrofuran (3 mL) and methanol (0.5 mL). After stirring for 45 min a clear solution resulted. The mixture was concentrated after 3 h and addition of ether and petroleum ether gave needles of **25**, R_f 0.45 (*B*), 0.88 (*F*); mp 180°C. *Anal.* calcd. for $C_{15}H_{14}BrNO_4$ (352.19): C 49.43, H 4.15, N 4.12; found: C 49.43, H 4.10, N 3.91.

*4-(4-Bromobenzyloxy)-3,5-diiodo-1-methyl-2(1H)-pyridone (**23**)*

The combined chloroform extracts of the residue, which were described in the preparation of **24** were concentrated and chromatographed on a column of silicic acid (8 g) with chloroform as mobile phase. Fractions containing the spot with R_f 0.45 (*C*) were pooled and evaporated. The residue was further purified by preparative tlc with the same solvent system. The silicic acid was extracted with system *G* and the product crystallized from diethyl ether – petroleum ether to give slightly yellow needles (29 mg), R_f 0.75 (*B*); mp 204–205°C; nmr ($CDCl_3$) δ: 3.58 (s, 3, NMe), 5.02 (s, 2, CH_2), 7.49, 7.53 (AA′BB′, 4, J_0 = 8.5 Hz, C_6H_4), and 7.65 (s, 1, H6). *Mol. Wt.* calcd. for $C_{13}H_{10}BrI_2NO_2$: 544.7989; found (ms) *m/e*: 544.8033.

*(6S,7R,2E,4E)-N-(7-Formyl-6-methoxy-5-methyl-2,4-octadienyl)acetamide (**34**)*

A solution of sodium metaperiodate (1.9 g) in water (160 mL) was added to a solution of **22** acetate (1 g) in a mixture of dioxane (140 mL) and methanol (20 mL). A transient wine-red color developed immediately indicating the liberation of iodine. The mixture was stirred in the dark for 2.5 h and centrifuged after addition of barium hydroxide to pH 8.3. Acetic anhydride (15 mL) was added to the stirred supernatant and, after one hour, the pH was adjusted to 8.4 with 19 *M* sodium hydroxide solution and extracted with ether (400, 200, and 200 mL). The combined and dried extracts were evaporated to a syrup (350 mg) of nearly pure **34**. Preparative tlc (*B*) gave a straw-colored syrup with R_f 0.55 (*B*) and 0.68 (*G*); cd (*c* 0.012 *M*, dioxane): $[\theta]_{318}$ 0, $[\theta]_{290}$ + 3750, $[\theta]_{248}$ 0, $[\theta]_{220}$ + 8750, $[\theta]_{204}$ 0; nmr ($CDCl_3$) δ: 0.85 (d, 3, $J_{2,8}$ = 7 Hz, H8), 1.69 (s, 3, 5-Me), 2.00 (s, 3, CH_3CO), 2.59 (ddq, 1, $J_{7,CHO}$ = 3, $J_{6,7}$ = 10, and $J_{7,8}$ = 7 Hz, H7), 3.17 (s, 3, OCH_3), 3.63 (d, 1, $J_{6,7}$ = 10 Hz, H6), 3.95 (t, 2, $J_{1,2}$ = $J_{1,NH}$ = 6 Hz, NCH_2), 5.67 (dt, 1, $J_{2,3}$ = 15 and $J_{1,2}$ = 6 Hz, H2), 6.00 (d, 1, $J_{3,4}$ = 10 Hz, H4), 6.06 (broad, 1, NH), 6.48 (dd, 1, $J_{3,4}$ = 10 and $J_{2,3}$ = 15 Hz, H3), and 9.75 (d, 1, $J_{7,CHO}$ = 3 Hz, 7-CHO).

*(2E,4E,6E)-8-[4-(4-Bromobenzyloxy)-1,2-dihydro-1-methyl-2-oxo-3-pyridyl]-7-methyl-8-oxo-2,4,6-octatrienoic Acid (**31**)*

The aqueous phase obtained after extraction of **34** in the above procedure was adjusted to pH 3.0 with 18 *N* sulfuric acid and the solution repeatedly extracted with dichloromethane. The dried extract was evaporated and the residue redissolved in chloroform. Upon standing the concentrated chloroform solution deposited a monochloroform solvate of **31** as yellow needles. Drying at 75°C and 0.5 Torr gave the chloroform-free product, R_f 0.15 (*A*) and 0.58 (*J*). *Anal.* calcd. for $C_{22}H_{20}BrNO_5$ (458.31): C 57.66, H 4.40, N 3.06, Br 17.43; found: C 57.71, H 4.36, N 3.18, Br 17.47 (40).

The ^1H nmr spectrum of **31** and a stereoscopic view of the crystal structure are shown in Figs. 3 and 4, respectively.

*(2E,4E,6E)-8-[4-(4-Bromobenzyloxy)-1,2-dihydro-1-methyl-2-oxo-3-pyridyl]-7-methyl-8-oxo-2,4,6-octatrienoic Acid Methyl Ester (**32**)*

A suspension of **31** (350 mg) in tetrahydrofuran (30 mL) and methanol (2 mL) was mixed with diethyl ether (30 mL) containing an excess of diazomethane and stirred overnight. The resulting clear solution was evaporated. Crystallization from methanol–water gave **32** as yellow needles, R_f 0.62 (*J*) and 0.75 (*F*); mp 164°C; nmr (DMSO-d_6) δ: 1.98 (s, 3, 7-Me), 3.43 (s, 3, NMe), 3.73 (s, 3, OMe), 5.19 (s, 2, CH_2), 6.11 (d, 1, $J_{2,3}$ = 15 Hz, H2), 6.41 (d, 1, $J_{5',6'}$ = 7.5 Hz, H5′), 6.74 (dd, 1, $J_{3,4}$ = 11 and $J_{4,5}$ = 14.5 Hz, H4), 6.86 (d, 1, $J_{5,6}$ = 11 Hz, H6), 7.25 (dd, 1, $J_{4,5}$ = 14.5 and $J_{5,6}$ = 11 Hz, H5), 7.26, 7.54 (AA′BB′, 4, J_0 = 8.5 Hz, C_6H_4), 7.47 (dd, 1, $J_{2,3}$ = 15 and $J_{3,4}$ = 11 Hz, H3), and 7.84 (d, 1, $J_{5',6'}$ = 7.5 Hz, H6′). *Anal.* calcd. for $C_{23}H_{22}BrNO_5$ (472.34): C 58.49, H 4.69, N 2.97; found: C 58.17, H 4.86, N 2.93.

*(6S,7S,2E,4E)-N-(8-Hydroxy-5,7-dimethyl-6-methoxy-2,4-octadienyl)acetamide (**37**)*

A solution of **34** (100 mg) in ethanol (10 mL) was cooled in an ice bath and treated with sodium borohydride (100 mg). After 20 min at 0°C water (20 mL) was added and the pH of the solution was adjusted to 3.1. The mixture was extracted with diethyl ether (3 × 25 mL). The dried extracts were evaporated and the residue purified by preparative tlc in system *B* to yield a colorless syrup of **37** (90 mg), R_f 0.48 (*B*) and 0.59 (*G*); uv (2-propanol) λ_{max}: 239 (ε 21 500) nm; nmr ($CDCl_3$) δ: 0.67 (d, 3, $J_{6,7}$, $J_{7,Me}$ = 6.5 Hz, 7-Me), 1.66 (s, 3, 5-Me), 1.94 (m, 1, H7), 1.98 (s, 3, CH_3CO), 3.16 (broad, 1, OH), 3.16 (s, 3, 6-OMe), 3.34 (d, 1, $J_{6,7}$ = 9 Hz, H6), 3.60 (d, 2, $J_{7,8}$ = 6 Hz, H8), 3.91 (t, 2, $J_{1,2}$ = $J_{1,NH}$ = 6 Hz, NCH_2), 5.67 (dt, 1, $J_{2,3}$ = 15 and $J_{1,2}$ = 6 Hz, H2), 5.96 (d, 1, $J_{3,4}$ = 11 Hz, H4), 6.37 (d, 1, $J_{1,NH}$ = 6 Hz, NH), and 6.42 (dd, 1, $J_{3,4}$ = 11 and $J_{2,3}$ = 15 Hz, H3).

*(2S,3S,4E,6E)-8-Acetylamino-3-methoxy-2,4-dimethyl-4,6-octadien-1-yl 4-Bromobenzoic Acid Ester (**38**)*

The ester was prepared with 4-bromobenzoyl chloride in pyridine in the usual fashion yielding **38** as a syrup, R_f 0.73 (2); nmr ($CDCl_3$) δ: 0.86 (d, 3, $J_{2,Me}$ = 7 Hz, 2-Me), 1.68 (s, 3, 4-Me), 2.00 (s, 3, CH_3CO), 2.09 (m, 1, H2), 3.14 (s, 3, 3-OMe), 3.31 (d, $J_{2,3}$ = 9 Hz, H3), 3.94 (t, 2, $J_{7,8}$ = $J_{8,NH}$ = 6 Hz), 4.30 (dd, 1, J_{gem} = 11 Hz, $J_{1a,2}$ = 6.5 Hz, H1a), 4.54 (dd, J_{gem} = 11 Hz, $J_{1b,2}$ = 4 Hz, H1b), 5.66 (dt, 1, $J_{6,7}$ = 15.5 and $J_{7,8}$ = 6 Hz, H7), 5.96 (d, 1, $J_{5,6}$ = 10.5 Hz, H5), 5.98 (broad, 1, NH), 6.44 (dd, 1, $J_{5,6}$ = 10.5 and $J_{6,7}$ = 15.5 Hz, H6), and 7.57, 7.92 (AA′BB′, 4, J_0 = 8.5 Hz, C_6H_4).

*(2R,3S,4E,6E)-8-[(2,4-Dinitrophenyl)amino]-3-methoxy-2,4-dimethyl-4,6-octadienal (**35**)*

A solution of sodium metaperiodate (1.5 g) in water (160 mL) was added to a solution of **22** acetic acid salt (1 g) in dioxane (140 mL) and methanol (20 mL). After standing in the dark for 5 h the pH was adjusted to 8.4 (barium hydroxide) and the suspension was centrifuged. Sodium hydrogen carbonate (1.5 g) was added to the supernatant, followed by 2,4-dinitrofluorobenzene (1 g) in methanol (5 mL). After stirring in the dark for 16 h, the mixture was extracted with diethyl ether and the extracts were combined, dried, and evaporated. A chloroform solution of the residue was chromatographed on silicic acid (75 g) with chloroform as mobile phase. The major yellow band was isolated and gave **35** as a yellow syrup (270 mg), R_f 0.67 (*D*); ^1H nmr shown in Fig. 5. *Mol. Wt.* calcd. for $C_{17}H_{21}N_3O_6$: 363.14, found (ms) *m/e* (%): 363 (6), 196 (100).

*(2R,3S,4E,6E)-8-[(2,4-Dinitrophenyl)amino]-3-methoxy-2,4-dimethyl-4,6-octadienal Dimethyl Acetal (**41**)*

To a solution of **35** (110 mg) in methanol (5 mL) and 2,2-

dimethoxypropane (1 mL) was added bis(4-nitrophenyl)phosphate (50 mg). After two h at room temperature, the solution was neutralized with Dowex 1 (HCO_3^-). The filtrate and resin washings (acetone) were concentrated, redissolved in chloroform, and chromatographed on a column of silicic acid (35 g) with chloroform as mobile phase. The major band was concentrated to a syrup which solidified on standing. Recrystallization from acetone–ethanol gave yellow prisms of **41**, R_f 0.78 (*D*); nmr ($CDCl_3$) δ: 0.71 (d, 3, $J_{2,Me}$ = 7 Hz, 2-Me), 1.68 (s, 3, 4-Me), 1.97 (m, 1, H2), 3.17 (s, 3, 3-OMe), 3.40 (d, $J_{2,3}$ = 9 Hz, H3), 3.43, 3.50 (2s, 3 each, 1-$(OMe)_2$), 4.17 (t, 2, $J_{7,8}$ = $J_{8,NH}$ = 6 Hz, NCH_2), 4.53 (d, 1, $J_{1,2}$ = 3 Hz, H1), 5.77 (dt, $J_{6,7}$ = 15 and $J_{7,8}$ = 6 Hz, H7), 6.04 (d, 1, $J_{5,6}$ = 10.5 Hz, H5), 6.60 (dd, 1, $J_{5,6}$ = 10.5 and $J_{6,7}$ = 15 Hz, H6), 6.95 (d, 1, $J_{5',6'}$ = 9.5 Hz, H6′), 8.30 (dd, 1, $J_{3',5'}$ = 2.5 and $J_{5',6'}$ = 9.5 Hz, H5′), 8.66 (broad, 1, NH), 9.16 (d, 1, $J_{3',5'}$ = 2.5 Hz, H3′).

*(2*R*,3*S*,4*E*,6*E*)-8-[(3-Bromo-4,6-dinitrophenyl)amino]-3-methoxy-2,4-dimethyl-4,6-octadienal Dimethyl Acetal (**42**)*

A solution of sodium metaperiodate (1.9 g) in water (70 mL) was added to a solution of **22** acetic acid salt (500 mg) in dioxane (70 mL) and methanol (10 mL). After standing for 4 h in the dark, the mixture was adjusted to pH 8.3 with barium hydroxide solution and centrifuged. Sodium hydrogen carbonate (1.5 g) and 1-bromo-5-fluoro-2,4-dinitrobenzene (42) (1 g) were added, the mixture stirred in the dark for 3 h and stored overnight at 4°C. The octadienal **36** was isolated and converted to the dimethyl acetal **42** as described (42), R_f 0.75 (*D*) and 0.24 (*H*); nmr ($CDCl_3$) δ: 0.71 (d, 3, $J_{2,Me}$ = 7 Hz), 1.68 (s, 3, 4-Me), 1.97 (m, 1, H2), 3.17 (s, 3, 3-OMe), 3.39 (d, 1, $J_{2,3}$ = 9 Hz, H3), 3.43, 3.49 (2s, 3 each, 1-$(OMe)_2$), 4.10 (t, 2, $J_{7,8}$ = $J_{8,NH}$ = 6 Hz, NCH_2), 4.50 (d, 1, $J_{1,2}$ = 3 Hz, H1), 5.71 (dt, $J_{6,7}$ = 15 and $J_{7,8}$ = 6 Hz, H7), 6.02 (d, 1, $J_{5,6}$ = 10.5 Hz, H5), 6.60 (dd, 1, $J_{5,6}$ = 10.5 and $J_{6,7}$ = 15 Hz, H6), 7.21 (s, 1, H2′), 8.43 (broad, 1, NH), and 8.98 (s, 1, H5′).

*[3a*R*,4*S*,6*R*,6a*S*,6(*S**),7(*R**)]-N[6-[7-[(4-Bromobenzyloxy)-1,2-dihydro-1-methyl-2-oxo-3-pyridinyl]-6-methyl-7-oxo-1(*E*),3(*E*),5(*E*)-heptatrienyl]tetrahydrofuro-[3,4-d]-2,2-dimethyl-1,3-dioxol-4-yl]-6-methoxy-5,7-dimethyl-2(*E*),4(*E*)-heptadienyl]acetamide (**60**)*

Bis(4-nitrophenyl)phosphate (0.5 g) was added to a solution of *N*-acetyl **22** (2 g) in acetone (40 mL) and 2,2-dimethoxypropane (15 mL). The solution was neutralized the next day with Dowex 1-X2 (HCO_3^-), filtered, and evaporated. The resulting syrup was chromatographed on a column of Sephadex LH-20 (51 mm × 510 mm) with acetone as mobile phase to yield homogeneous **60** as yellow powder upon concentration to dryness (1.47 g), R_f 0.35 (*B*) and 0.60 (*G*); nmr ($CDCl_3$)[5] δ: 0.90 (d, 3, $J_{7,Me}$ = 7 Hz, 7-Me), 1.28, 1.42 (2s, 3 each, 2′-Me_2), 1.66 (s, 3, 5-Me), 1.97 (s, 3, 6″-Me), 1.99 (s, 3, $COCH_3$), 2.29 (m, 1, H7), 3.13 (s, 3, 6-OMe), 3.29 (d, 1, $J_{6,7}$ = 9 Hz, H6), 3.45 (s, 3, NMe), 3.48 (broad, 1, H4′), 3.87 (t, 2, $J_{1,2}$ = $J_{1,NH}$ = 6 Hz, NCH_2), 3.90 (broad, 1, H6′), 4.62 (m, 2, H3a′ and H6a′), 5.04 (s, 1, OCH_2), 5.64 (dt, 1, $J_{2,3}$ = 15 and $J_{1,2}$ = 6 Hz, H2), 5.92 (m, 3, H4, H1″ and NH), 6.04 (d, 1, $J_{5''',6'''}$ = 7.5 Hz, H5‴), 6.49 (m, 4, H3, H2″, H3″, H4″), 6.87 (d, 1, $J_{4'',5''}$ = 10 Hz, H5″), 7.30 (d, 1, $J_{5''',6'''}$ = 7.5 Hz, H6‴), and 7.10, 7.43 (AA′BB′, 4, J_0 = 8.5 Hz, C_6H_4).

*[3a*R*,4*S*,6*S*,6a*R*,4(*S**),5(*R**)]-5-[6-Formyltetrahydro-2,2-dimethylfuro[3,4-d]-1,3-dioxol-4-yl]-4-methoxy-3,5-dimethyl-2-pentenal (**61**), [3a*R*,4*S*,6*S*,6a*R*,1(*R**),2(*S**)]-Tetrahydro-6-(2-methoxy-1-methyl-3-oxobutyl)furo[3,4-d]-1,3-dioxole-4-carboxaldehyde (**62**), and 4-(4-Bromobenzyloxy)-1-methyl-3-pyruvoyl-2(1*H*)-pyridone (**28**)*

A solution of osmium tetroxide (102 mg) in dioxane–water (1:1, 160 mL) was combined with a solution of **60** (6.3 g, 8 mmol) in dioxane (160 mL). Sodium metaperiodate (71 g, 0.33 mol) was added in portions to the stirred solution over a one hour period. After three additional hours of stirring in the dark the mixture was filtered and the filtrate extracted continuously with diethyl ether for six hours. The dried extract was evaporated to a syrup (6.2 g) which was chromatographed on silicic acid (350 g) taking fractions of 15 mL and using chloroform (fractions 1–120), chloroform – 2-propanol (99:1, fractions 121–240), and chloroform–methanol (9:1, fractions 241–360) as mobile phases.

Fractions 206–216 contained a mixture of **61** as major and **62** as minor component and was obtained as a colorless syrup (1.5 g).

Fractions 218–236 gave a crystalline residue, which was recrystallized from methanol as colorless needles of **28** (0.68 g), R_f 0.06 (*C*) and 0.72 (*G*, blue tetrazoleum) (40).

Fractions 260–300 furnished **24** as white needles from aqueous ethanol, R_f 0.10 (*A*).

The mixture of **61** and **62** was partially resolved by chromatography on silicic acid (85 g) with chloroform as mobile phase taking 15 mL fractions. Fractions 36–48 gave a mixture of **61** and **62** in a ratio of 3:1 (0.60 g) and fractions 49–75 afforded pure **61** (0.57 g) as a colorless syrup, R_f 0.32 (*C*) and 0.86 (*G*, blue tetrazoleum); nmr ($CDCl_3$) δ: 1.09 (d, 3, $J_{5,Me}$ = 7 Hz, 5-Me), 1.27, 1.43 (2s, 3 each, 2′-Me_2), 2.15 (d, 3, $J_{2,Me}$ = 1.5 Hz, 3-Me), 2.47 (sextet, $J_{4,5}$ = $J_{4',5}$ = $J_{5,Me}$ = 7 Hz, H5), 3.26 (s, 3, 4-OMe), 3.49 (d, 1, $J_{4,5}$ = 7 Hz, H4), 3.62 (dd, 1, $J_{3a',4'}$ = 3.5 and $J_{4',5}$ = 7 Hz, H4′), 3.87 (dd, 1, $J_{6',6a'}$ = 4 and $J_{6',CHO}$ = 1.5 Hz, H6′), 4.68 (dd, 1, $J_{3a',4'}$ = 3.5 and $J_{3a',6a'}$ = 6 Hz, H3a′), 4.96 (ddt, 1, $J_{3a',6a'}$ = 6 and $J_{6',6a'}$ = 4 Hz, H6a′), 6.03 (d, 1, $J_{1,2}$ = 8 Hz, H2), 9.62 (d, 1, $J_{6',CHO}$ = 1.5 Hz, 6′-CHO), 10.08 (d, 1, $J_{1,2}$ = 8 Hz, H1).

The mixture of **61** and **62** (0.60 g) was dissolved in methanol (15 mL) and ozonized at −78°C for 30 min although ozone-uptake required only 5 min. Excess ozone was removed with nitrogen and dimethyl sulfide was added at room temperature until potassium iodide was no longer oxidized. The solution was concentrated to yield crude **62** without any remaining **61**. For further purification the residue was chromatographed on silicic acid (35 g) with chloroform as mobile phase yielding **62** (0.40 g) as the major detectable component with blue tetrazoleum, R_f 0.43 (*C*) and 0.90 (*G*); nmr ($CDCl_3$) δ: 1.11 (d, 3, $J_{1,Me}$ = 7 Hz, 1-Me), 1.25, 1.39 (2s, 3 each, 2-Me_2), 2.16 (s, 3, 3-Me), 2.56 (ddq, 1, $J_{1,2}$ = 9, $J_{1,6}$ = 5, and $J_{1,Me}$ = 7 Hz, H1), 3.35 (s, 3, 2-OMe), 3.44 (d, 1, $J_{1,2}$ = 5 Hz, H2), 3.58 (dd, 1, $J_{1,6}$ = 5 and $J_{6,6a}$ = 9 Hz, H6), 3.86 (dd, 1, $J_{3a,4}$ = 4.5 and $J_{4,CHO}$ = 1.5 Hz, H4), 4.22 (dd, 1, $J_{3a,6a}$ = 6 and $J_{6,6a}$ = 3.5 Hz, H6a), 4.46 (dd, 1, $J_{3a,4}$ = 4.5 and $J_{3a,6a}$ = 6 Hz, H3a), and 9.57 (d, 1, $J_{4,CHO}$ = 1.5 Hz, 4-CHO).

*[1*R*,3*R*,4*S*,6*S*,7*S*,8*S*,10*R*]-4-(Dimethoxymethyl)-1,7-dimethyl-8-methoxy-2,5,9-trioxatricyclo[4.2.2.0^{3,10}]decane(**64***b*), [2*S*-(2β,3β,3aα,5β,6α,6aα)]-Hexahydro-5-methoxy-2-(dimethoxymethyl)-6-methylfuro[3,2-b]furan-3-ol (**65***a*), and [2*S*-(2β,3β,3aα,5α,6α,6aα)]-Hexahydro-5-methoxy-2-(dimethoxymethyl)-6-methylfuro[3,2-b]furan-3-ol(**65***b*)*

A solution of crude **62** (560 mg) in methanol (10 mL) was cooled to −78°C and acetyl chloride (1 mL) was added. The mixture was allowed to warm to 23°C, kept for 3 h at 23°C, and then refrigerated overnight. After the solution was neutralized with 2.25 *N* methanolic sodium methoxide (6.3 mL), cyclohexane (50 mL) and water (30 mL) were added. The aqueous phase was extracted twice more with cyclohexane. From the combined, water-washed cyclohexane extracts, **64***b* was obtained as colorless prisms and recrystallized from petroleum ether (80 mg), mp 98°C (49). Additional **64***b* could be obtained by chromatography of the mother liquors on silicic acid with system *I* as mobile phase, R_f 0.67 (*C*) (sulfuric acid spray); nmr ($CDCl_3$) δ: 0.94 (d, 3, $J_{7,Me}$ = 7.5 Hz, 7-Me), 1.49 (s, 3, 1-Me), 2.40 (d quintet, 1, $J_{6,7}$ = 2 and $J_{7,8}$ = $J_{7,Me}$ = 7.5 Hz, H7), 3.36, 3.43, 3.48 (3s, 3 each, 3-OMe), 3.40 (d, 1, $J_{7,8}$ = 7.5 Hz, H8), 3.75

(dd, 1, $J_{3,4}$ = 2 and $J_{4,CH}$ = 7.5 Hz, H4), 4.20 (dd, 1, $J_{6,7}$ = 2 and $J_{6,10}$ = 7.5 Hz, H6), 4.25 (dd, 1, $J_{3,4}$ = 2 and $J_{3,10}$ = 4 Hz, H3), 4.53 (d, 1, $J_{4,CH}$ = 7.5 Hz, 4-CH), 5.02 (dd, 1, $J_{3,10}$ = 4 and $J_{6,10}$ = 7.5 Hz, H10).

The aqueous phase remaining after the extraction with cyclohexane was extracted twice with chloroform to yield a residue which was chromatographed on silicic acid (10 g) collecting 10-mL fractions and using system *I* as mobile phase. Fractions 24–26 gave pure **65***b* as colorless syrup (30 mg), R_f 0.48 (*C*, sulfuric acid); nmr ($CDCl_3$) δ: 1.05 (d, 3, $J_{6,Me}$ = 7 Hz, 6-Me), 2.29 (ddq, $J_{5,6}$ = 4.5, $J_{6,6a}$ = 5.5 and $J_{6,Me}$ = 7 Hz, H6), 2.76 (broad, 1, OH), 3.35, 3.41, 3.47 (3s, 3 each, 3 OMe), 3.73 (dd, 1, $J_{2,3}$ = 3 and $J_{2,CH}$ = 7.5 Hz, H2), 4.10 (dd, 1, $J_{2,3}$ = 3 and $J_{3,3a}$ = 5.5 Hz, H3), 4.24 (dd, 1, $J_{3a,6a}$ = 6.5 and $J_{6,6a}$ = 5.5 Hz, H6a), 4.64 (d, $J_{2,CH}$ = 7.5 Hz, 2-CH), 4.64 (dd, 1, $J_{3,3a}$ = 5.5 and $J_{3a,6a}$ = 6.5 Hz, H3a), and 5.03 (d, $J_{5,6}$ = 4.5 Hz, H5).

Fractions 28–35 yielded pure **65***a* as colorless syrup (30 mg), R_f 0.43 (*C*, sulfuric acid); nmr ($CDCl_3$) δ: 1.03 (d, 3, $J_{6,Me}$ = 7.5 Hz, 6-Me), 2.48 (q, $J_{5,6}$ = $J_{6,6a}$ < 1 and $J_{6,Me}$ = 7.5 Hz, H6), 3.35 (m, 1, H2), 3.42, 3.46, 3.51 (3s, 3 each, 3 OMe, overlapping with OH), 4.06 (d, 1, $J_{3a,6a}$ = 6 Hz, H6a), 4.11 (dd, 1, $J_{2,3}$ = 3 and $J_{3,3a}$ = 6 Hz, H3), 4.66 (d, 1, $J_{2,CH}$ = $J_{2,CH}$ = 7.5 Hz, 2-CH), 4.77 (s, 1, H5), 4.92 (t, $J_{3a,5}$ = 6 Hz, H3a).

*[2S-(2β,3β,3aα,5β,6α,6aα)]-Hexahydro-5-methoxy-2-(dimethoxymethyl)-6-methylfuro[3,2-b]furan-3-ol 4-Bromophenylcarbamic Acid Ester (**66**a)*

A mixture of **65***a* (25 mg), pyridine (1.5 mL), benzene (1.5 mL), and 4-bromophenylisocyanate (35 mg) was allowed to react overnight, diluted with cyclohexane (3 mL), and filtered. The filtrate was evaporated and purified by preparative tlc, and the band with R_f 0.22 (*C*) was extracted with system *G* to afford a crystalline residue of **66***a* which was recrystallized from benzene–cyclohexane (21 mg), mp 165°C. $[\alpha]_D$ −50.5° (*c* 0.5, ethanol); nmr ($CDCl_3$) δ: 1.07 (d, 3, $J_{6,Me}$ = 7.5 Hz, 6-Me), 2.43 (tq, 1, $J_{5,6}$ = $J_{6,6a}$ = 3 and $J_{6,Me}$ = 7.5 Hz, H6), 3.28, 3.37, 3.43 (3s, 3 each, 3 OMe), 3.90 (dd, 1, $J_{2,3}$ = 4 and $J_{2,CH}$ = 7.5 Hz, H2), 4.17 (dd, 1, $J_{3a,6a}$ = 6 and $J_{6,6a}$ = 3 Hz, H6a), 4.66 (d, 1, $J_{2,CH}$ = 7.5 Hz, 2-CH), 4.72 (d, 1, $J_{5,6}$ = 3 Hz, H5), 4.88 (t, 1, $J_{3,3a}$ = 5.5 and $J_{3a,6a}$ = 6 Hz, H3a), 5.21 (dd, 1, $J_{2,3}$ = 4 and $J_{3,3a}$ = 5.5 Hz, H3), 6.89 (s, 1, NH), and 7.34, 7.40 (AA′BB′, 4, J_0 = 8.5 Hz, C_6H_4). *Anal.* calcd. for $C_{18}H_{24}BrNO_7$ (446.30): C 48.44, H 5.42, N 3.14; found: C 48.56, H 5.52, N 3.21.

A crystal of **66***a* with the dimensions of 0.01 mm × 0.08 mm × 0.35 mm served for crystallographic analysis. It was of space group $P2_1$ with unit-cell dimensions a = 5.245(6), b = 13.981(10), c = 13.980(20) Å, β = 94.85(8)° and d calcd. = 1.449 g/cm³ for Z = 2. Of the 1020 reflections examined (2θ < 96.5°) only 520 were considered significant due to the small size of the crystal. The structure was solved by Patterson and Fourier methods with final refinement by full-matrix least squares. Anisotropic thermal parameters were used for the bromine atom and isotropic temperature factors were used for the lighter atoms; the hydrogen atoms were not included. The final discrepancy index is R = 0.082 (Fig. 11). Attempts to determine the absolute configuration of **66***a* on the basis of the available crystallographic data gave inconclusive results.

*[2S,(2β,3β,3aα,5α,6α,6aα)]-Hexahydro-5-methoxy-2-(dimethoxymethyl)-6-methylfuro[3,2-b]furan-3-ol 4-Bromophenylcarbamic Acid Ester (**66**b)*

The alcohol **65***b* was converted to the urethane **66***b*, purified by preparative tlc (R_f 0.53, *C*) as described for the anomer, and obtained as colorless needles from benzene–cyclohexane, mp 156°C; $[\alpha]_D$ −129.9° (*c* 0.5, ethanol); nmr ($CDCl_3$) δ: 1.07 (d, 3, $J_{6,Me}$ = 7.5 Hz, 6-Me), 2.32 (tq, 1, $J_{5,6}$ = $J_{6,6a}$ = 4.5 and $J_{6,Me}$ = 7.5 Hz, H6), 3.32, 3.37, 3.42 (3s, 3 each, 3 OMe), 4.00 (dd, 1, $J_{2,3}$ = 4.5 and $J_{2,CH}$ = 7.5 Hz, H2), 4.31 (dd, 1, $J_{3a,6a}$ = 6.5 and $J_{6,6a}$ = 4.5 Hz, H6a), 4.64 (d, 1, $J_{2,CH}$ = 7.5 Hz, 2-CH), 4.79 (dd, 1, $J_{3,3a}$ = 6 and $J_{3a,6a}$ = 6.5 Hz, H3a), 4.95 (d, 1, $J_{5,6}$ = 4.5 Hz, H5), 5.28 (dd, 1, $J_{2,3}$ = 4.5 and $J_{3,3a}$ = 6 Hz, H3), 6.70 (s, 1, NH), and 7.33, 7.41 (AA′BB′, 4, J_0 = 8.5 Hz, C_6H_4). *Anal.* calcd. for $C_{18}H_{24}BrNO_7$ (446.30): C 48.44, H 5.42, N 3.14; found: C 48.52, H 5.53, N 2.98.

Diffraction data of **66***b* were collected from a crystal with the dimensions of 0.15 mm × 0.30 mm × 0.45 mm, space group $P2_1$, and unit-cell dimensions of a = 11.371(5), b = 8.965(5), c = 10.116(5) Å, β = 94.66(4)° and d calcd. = 1.441 g/cm³ for Z = 2. Of the 2286 accessible reflections with 2θ < 152°, 2011 were considered significant. The structure was solved by the heavy atom method. Four Fouriers, based on successively more complete trial structures, were required to locate all non-hydrogen atoms. During the preliminary refinement of the structures the imaginary part of the anomalous dispersion correction ($\Delta f''$) was set to zero. At the conclusion of this refinement, in which all atoms were anisotropic, the positions of the hydrogen atoms were calculated. The hydrogen atoms were included in all subsequent calculations but their parameters were not refined. The final refinement of the structure was carried out by full-matrix least squares. In order to establish the absolute configuration, two separate refinements were made. In one the correct value of the imaginary part of the dispersion correction was used and in the other the sign of $\Delta f''$ was reversed (equivalent to refining the antipode of **66***b*). The absolute configuration was taken as the one which had the lower weighted R-value (0.0529 and 0.0572). The configuration corresponding to the higher R-value could be rejected (as being the correct absolute configuration) at a significance level substantially better than 0.005, according to the test described by Hamilton (57). The final unweighted discrepancy index is R = 0.037 for the 2011 observed reflections (Fig. 11).

*Mocimycin Methyl Ether (N-Desmethyl-**17**) and Aurodox Methyl Ether (**17**)*

Methyl iodide (2 mL) was added to a solution of mocimycin sodium salt (3 g) in dimethylformamide (15 mL) and the mixture kept in the dark for 4 days. The mixture was then equilibrated with ethyl acetate (250 mL) and water (50 mL). The ethyl acetate phase was washed with water (2 × 50 mL), 0.5 *M* trisodium phosphate solution (6 × 50 mL), water (5 × 50 mL), dried, and evaporated to afford an amorphous, yellow powder (1.7 g) which proved to be a mixture of two major components. Upon chromatography of this mixture on a column of Sephadex LH-20 (55 mm × 720 mm) with acetone as mobile phase, **17** (8) was eluted first (R_f 0.76, *A*) and obtained as yellow powder upon evaporation of the pooled fraction (0.4 g), rechromatography (Sephadex LH-20, 2-butanone) and precipitation from acetone – petroleum ether.

Continued elution furnished *N*-desmethyl-**17** (8), obtained as yellow powder (0.6 g) after rechromatography (Sephadex LH-20, 2-butanone) and precipitation from acetone – petroleum ether. Additional *N*-desmethyl-**17** (0.3 g) was obtained from aqueous extracts of the combined trisodium phosphate phases after chromatographic purification.

Compound **17** could also be prepared directly from **1***b*. A solution of **1***b* sodium salt in dimethylformamide and methyl iodide (10 mL) was shaken for 4 days in the dark and poured into a separatory funnel containing ethyl acetate (75 mL) and 5% aqueous sodium hydrogen carbonate solution (25 mL).

The organic phase was washed twice with sodium hydrogen carbonate solution, three times with brine, dried, filtered, and evaporated yielding crude **17** (4.59 g). A portion (1.5 g) was chromatographed on a column of Sephadex LH-20 (51 mm × 510 mm) with acetone as mobile phase. Homogeneous fractions (tlc) were pooled and evaporated to afford **17** as yellow, amorphous powder (1.0 g) (8).

[2R,3R,4S,5S,1(R),2(S*)]-4-Methoxy-3-[7-[5-[7-amino-2-methoxy-1,3-dimethyl-3(E),5(E)-heptadienyl]-3,4-dihydroxytetrahydro-2-furyl]-2-methyl-1-oxo-2(E),4(E),6(E)-heptatrienyl]-2(1H)-pyridinone Acetic Acid Salt (**45** Acetic Acid Salt)*

A solution of mocimycin methyl ether (*N*-desmethyl-**17**, 0.6 g) in acetic acid (6 mL) was heated on the steam bath for 30 min. After evaporation to dryness, the residue was chromatographed on a column of silicic acid (30 g) with chloroform (100 mL), chloroform – 2-propanol (19:1, 350 mL), and chloroform–methanol – acetic acid (15:4:1) as mobile phases. From the pooled and concentrated fractions *N*-desmethylgoldinamine methyl ether acetic acid salt was obtained as amorphous, yellow powder by freeze-drying (0.2 g), R_f 0.45 (*A*); nmr (CD_3OD)[8] δ: 0.82 (d, 3, $J_{1,Me}$ = 7 Hz, 1-Me), 1.73 (s, 3, 3-Me), 1.91 (s, 3, OAc), 1.97 (s, 3, 2″-Me), 2.16 (m, 1, H1), 3.17 (s, 3, 2-OMe), 3.38 (s, 1, $J_{1,2}$ = 9.5 Hz, H2), 3.66 (m, 3, NCH_2 and H5′), 3.84 (s, 3, 4‴-OMe), 4.19 (m, 1, H4′), 4.30 (m, 2, H2′ and H3′), 5.97 (m, 3, H4, H6, H7″), 6.45 (d, 1, $J_{5''',6'''}$ = 7.5 Hz, H5‴), 6.30–6.80 (m, 4, H4″, H5, H5″, H6″), 6.96 (d, 1, $J_{3'',4''}$ = 10 Hz, H3″), and 7.55 (d, 1, $J_{5''',6'''}$ = 7.5 Hz, H6‴).

[2S,3S,4R,5R,6(S),7(R*)]-N[7-[5-[7-(1,2-Dihydro-4-methoxy-2-oxo-3-pyridinyl)-6-methyl-7-oxo-1(E),3(E),5(E)-heptatrienyl]tetrahydro-3,4-dihydroxyfuran-2-yl]-6-methoxy-5,7-dimethyl-2(E),4(E)-heptadienyl]trifluoroacetamide (**46**)*

To a solution of **45** acetic acid salt (71 mg) in 1 mL of methanol containing triethylamine (0.1 mmol/mL) was added trifluoroethanethioic acid *S*-ethyl ester (25 μL). The mixture was concentrated after 18 h and purified by preparative tlc (R_f 0.25, acetone) to afford amorphous **46** as yellow powder; ord (*c* 0.35, dioxane): $[\phi]_{700}$ −257° (start), $[\phi]_{589}$ −426°, $[\phi]_{344}$ −14 190° (trough), $[\phi]_{328}$ 0° (intersects), $[\phi]_{308}$ +14 190° (peak), $[\phi]_{282}$ +9 310 (trough), $[\phi]_{252}$ +28 380° (peak), $[\phi]_{246}$ 0° (intersects), $[\phi]_{215}$ −31 930° (trough), $[\phi]_{212}$ −29 710° (last); cd (0.006 *M*, dioxane): $[\theta]_{380}$ 0 (start), $[\theta]_{329}$ −17 425, $[\theta]_{289}$ −2 010, $[\theta]_{265}$ −3 350, $[\theta]_{257}$ 0 (intersects), $[\theta]_{234}$ +29 320, $[\theta]_{210}$ 0 (last). *Mol. Wt.* calcd. for $C_{30}H_{37}F_3N_2O_8$: 610.63; found (ms) *m/e* (%): 610 (0.3), 152 (1,2-dihydro-4-methoxy-2-oxo-3-carbonyl cation, 100).

[2R,3R,4S,5S,1(R),2(S*)]-4-Methoxy-1-methyl-3-[7-[5-[7-amino-2-methoxy-1,3-dimethyl-3(E),5(E)-heptadienyl]-3,4-dihydroxytetrahydro-2-furyl]-2-methyl-1-oxo-2(E),4(E),6(E)-heptatrienyl]-2(1H)-pyridone Acetic Acid Salt (**21** Acetic Acid Salt)*

A solution of **17** (2.35 g) in acetic acid (20 mL) was either allowed to stand at room temperature in the dark for 4.5 days or was heated on the steam bath for 20 min, the solvent was evaporated, and the residue subjected to Craig distribution in chloroform–methanol – acetic acid – water (5:1:1:4). After 199 transfers, tubes 145–177, which contained a homogeneous solute, were pooled, concentrated, and freeze-dried, yielding goldinamine methyl ether acetic acid salt (**21** acetic acid salt) as amorphous, yellow powder (0.97 g), R_f 0.64 (*A*); nmr ($CDCl_3$) δ: 0.84 (d, 3, $J_{1,Me}$ = 7 Hz, 1-Me), 1.67 (s, 3, 3-Me), 2.00 (s, 6, OAc and 2″-Me), 2.17 (m, 1, H1), 3.17 (s, 3, 2-OMe), 3.25 (d, 1, $J_{1,2}$ = 9.5 Hz, H2), 3.49 (s, 3, NMe), 3.49 (broad, 3, NCH_2 and H5′), 3.76 (s, 3, 4″-OMe), 4.17 (m, 1, H2′), 4.34 (m, 2, H3′ and H4′), 5.97 (m, 3, H4, H6, H7″), 6.10 (d, 1, $J_{5''',6'''}$ = 7.5 Hz, H5‴), 6.49 (m, 4, H4″, H5, H5″, H6″), 6.86 (d, 1, $J_{3'',4''}$ = 10 Hz, H3″), 7.38 (d, 1, $J_{5''',6'''}$ = 7.5 Hz, H6‴).

[8]Proton designations are those used in **22** (Scheme 6).

[2S,3S,4R,5R,6(S),7(R*)]-N-[7-[5-[7-(1,2-Dihydro-4-methoxy-1-methyl-2-oxo-3-pyridinyl)-6-methyl-7-oxo-1(E),3(E),5(E)-heptatrienyl]tetrahydro-3,4-dihydroxyfuran-2-yl]-6-methoxy-5,7-dimethyl-2(E),4(E)-heptadienyl]trifluoroacetamide (**47**)*

Goldinamine methyl ether acetic acid salt (**21** acetic acid salt, 105 mg) was converted to the *N*-trifluoroacetamide as described above in the preparation of **46**. The evaporated reaction product was chromatographed on a column of Sephadex LH-20 (acetone) to yield **47** as yellow powder (87 mg), R_f 0.23 (*B*). *Mol. Wt.* calcd. for $C_{31}H_{39}F_3N_2O_8$: 624.66; found (ms) *m/e* (%): 624 (0.4), 166 (1,2-dihydro-4-methoxy-1-methyl-2-oxo-3-carbonyl cation, 100).

[2S,3S,4R,5R,6(S),7(R*)]-N-[7-[5-[7-(1,2-Dihydro-4-methoxy-1-methyl-2-oxo-3-pyridinyl)-6-methyl-7-oxo-1(E),3(E),5(E)-heptatrienyl]tetrahydro-3,4-dihydroxyfuran-2-yl]-6-methoxy-5,7-dimethyl-2(E),4(E)-heptadienyl]acetamide (**49**)*

A solution of **21** acetic acid salt (200 mg) in methanol (1.5 mL) and acetic anhydride (0.7 mL) was kept overnight at 25°C and added to a mixture of ether (55 mL) and petroleum ether. The precipitate was collected by centrifugation, washed repeatedly, and chromatographed on a column of Sephadex LH-20 with 2-butanone as mobile phase. The *N*-acetyl compound, **49**, was obtained as amorphous, yellow powder (152 mg), R_f 0.53 (*A*). *Mol. Wt.* calcd. for $C_{31}H_{42}N_2O_8$: 570; found (ms) *m/e*: 570, 552 (M − H_2O), 538 (M − MeOH), 520 (M − H_2O − MeOH), and 506 (M − 2MeOH) as highest mass peaks. Calculated: 552.2840 ($C_{31}H_{40}N_2O_7$), 538.2683 ($C_{30}H_{38}N_2O_7$), 520.2577 ($C_{30}H_{36}N_2O_6$), 506.2420 ($C_{29}H_{34}N_2O_6$); found: 552.2844, 538.2701, 520.2566, 506.2429.

[2S,3R,4S,5R,6(S),7(R*)]-N-[7-[5-[7-(1,2-Dihydro-4-methoxy-1-methyl-2-oxo-3-pyridinyl)-6-methyl-7-oxo-1(E),3(E),5(E)-heptatrienyl]tetrahydro-3,4-dimethoxyfuran-2-yl]-6-methoxy-5,7-dimethyl-2(E),4(E)-heptadienyl]acetamide (**50**)*

To a solution of **49** (100 mg) in dimethylformamide (1 mL) was added barium oxide (100 mg) and barium hydroxide octahydrate (100 mg) followed by dimethylsulfate (0.2 mL) (58). After stirring for 24 h additional barium oxide (100 mg) and dimethylsulfate (0.1 mL) were added. After a total reaction time of 40 h concentrated ammonium hydroxide solution (0.2 mL) was added and stirring continued for 10 min. Addition of chloroform (10 mL) to the stirred mixture was followed by centrifugation. The chloroform solution was decanted and the syrupy solids were washed repeatedly with chloroform. The combined chloroform extracts were washed with brine (3 × 10 mL) and water (10 mL) and evaporated. The resulting solution of **50** in *N,N*-dimethylformamide was diluted with acetone and chromatographed on a column of Sephadex LH-20 (acetone). Pure **50** (30 mg) was isolated as usual, R_f 0.10 (*B*). *Mol. Wt.* calcd. for $C_{33}H_{46}N_2O_8$: 598.74; found (ms) *m/e*: 598, 566 (M − MeOH), and 534 (M − 2MeOH) as highest mass peaks. Calculated: 598.3259 ($C_{33}H_{46}N_2O_8$), 566.2996 ($C_{32}H_{42}N_2O_7$), 534.2734 ($C_{31}H_{38}N_2O_6$); found: 598.3326 (extrapolated over 30 mass units), 566.2979, 534.2728.

*(2E,4E,6E)-8-[4-Methoxy-1,2-dihydro-1-methyl-2-oxo-3-pyridinyl]-7-methyl-8-oxo-2,4,6-octatrienoic Acid (**29**)*

To a solution of **21** acetic acid salt (200 mg) in methanol (4 mL) and dioxane (16 mL) was added sodium metaperiodate (0.5 g) in water (20 mL). The mixture was stirred for 4 h at 25°C in the dark, the pH was adjusted to 8.4 with barium hydroxide and the

resulting suspension centrifuged. Acetic anhydride (1.5 mL) was added to the supernatant under stirring, followed by another 0.5 mL 30 min later. After one hour the mixture was adjusted to pH 8.4 with sodium hydroxide and repeatedly extracted with diethyl ether. Drying and evaporation of the combined ether phase gave **34** (50 mg). The aqueous phase was adjusted to pH 3 (sulfuric acid) and extracted with dichloromethane (2 × 40 mL). Evaporation gave a residue which crystallized from methanol–chloroform–ether to yield **29** as yellow prisms, R_f 0.13 (*A*), 0.52 (*J*); mp 220–222°C; uv λ_{max} (ε): 208–209 (32 500), inflection 232–233 (5 500), inflection 295 (16 000), 331–332 (41 200), and inflection 340 (39 900) nm in 0.1 *N* HCl, 208–209 (33 000), inflection 232 (8 000), inflection 295 (16 000), and 333–340 (39 600) nm in buffer (pH = 7), inflection 232 (7 800), inflection 295 (16 500), and 332–340 (40 000) nm in 0.1 *N* KOH; nmr (Me_2SO-d_6) δ: 1.91 (s, 3, 7-Me), 3.37 (s, 3, OMe), 3.72 (s, 3, NMe), 5.95 (d, 1, $J_{2,3}$ = 15 Hz, H2), 6.31 (d, 1, $J_{5',6'}$ = 7.5 Hz, H5′), 6.70 (dd, 1, $J_{3,4}$ = 11 and $J_{4,5}$ = 14.5 Hz, H4), 6.81 (d, 1, $J_{5,6}$ = 11 Hz, H6), 7.12 (dd, 1, $J_{4,5}$ = 14.5 and $J_{5,6}$ = 11 Hz, H5), 7.30 (dd, 1, $J_{2,3}$ = 15 and $J_{3,4}$ = 11 Hz, H3), and 7.79 (d, 1, $J_{5',6'}$ = 7.5 Hz, H6′). *Mol. Wt.* calcd. for $C_{16}H_{17}NO_5$: 303.33; found (ms) *m/e (%)*: 303 (17), 166 (100).

*[1R-(1β,2α,6α,8β)]-4,4-Dimethyl-3,5,7,11-tetraoxatricyclo-[6.3.0.0^{2,6}]undecan-9-one (**68**)*

To a vigorously stirred solution of 3,6-anhydro-1,2-*O*-isopropylidine-α-D-glucofuranose (**67**, 1.70 g, 8.41 mmol) (50) (R_f 0.07 (*H*), greenish color with anisaldehyde – sulfuric acid spray) in dichloromethane (50 mL) was added dropwise ca. 0.2 *M* sodium metaperiodate solution while keeping the pH at 6–7 with intermittent addition of sodium hydrogen carbonate solution (59). Addition was complete after 30 min as indicated by a permanent color change from dark to greenish yellow. Stirring was continued for 3 h, 1-propanol (0.5 mL) was added, and the mixture was filtered 15 min later. The dichloromethane phase was decanted, combined with two further extracts, washed twice with a little water, dried, and evaporated to yield **68** as a syrup. Short-path distillation (bath temperature 105°C, 0.5 Torr) gave a colorless syrup (1.45 g, 7.24 mmol, 86%) which crystallized very slowly on standing, R_f 0.06 (*H*), reddish color with anisaldehyde; ir (neat) ν: 1778 (ketone) cm^{-1}; nmr ($CDCl_3$) δ: 1.33, 1.48 (2s, 3 each, 4-Me_2), 3.90, 4.24 (AB, 2, $J_{10a,10b}$ = 17 Hz, H10), 4.40 (d, 1, $J_{1,8}$ = 3 Hz, H1), 4.71 (d, 1, $J_{1,8}$ = 3 Hz, H8), 4.75 (d, 1, $J_{1,2}$ = 0 and $J_{2,6}$ = 3.5 Hz, H2), 6.02 (d, 1, $J_{2,6}$ = 3.5 Hz, H6).

*[1S-(1β,2α,6α,8β)]-4,4-Dimethyl-9-methylene-3,5,7,11-tetraoxatricyclo[6.3.0.0^{2,6}]undecane (**69**)*

A hexane solution of *n*-butyllithium (3.75 mL, 1.6 *M*) was added to diethyl ether (15 mL) under argon followed by methyltriphenylphosphonium bromide (2.14 g, 6 mmol) in one portion under stirring. A solution was gradually obtained and after 4 h of continued stirring a solution of **68** (1 g, 5 mmol) in diethyl ether (15 mL) was added. The resulting suspension was refluxed under stirring for 3 h and stirred overnight at room temperature. The ethereal supernatant was combined with three additional ether extracts, washed with water, dried, and evaporated to a syrup. Dissolving the syrup in a little chloroform and adding petroleum ether deposited crystalline triphenylphosphine oxide. Evaporation of the mother liquor gave a syrup containing mostly **69** purified by chromatography on a column of silica gel with system *I* as mobile phase to give **69** as colorless syrup (0.249 g, 1.26 mmol), R_f 0.53 (*H*), brownish color with anisaldehyde – sulfuric acid spray; nmr ($CDCl_3$) δ: 1.34, 1.52 (2s, 3 each, 4-Me_2), 4.30, 4.54 (tAB, 2, $J_{6,7}$ = 2 and $J_{10a,10b}$ = 13 Hz, H10), 4.43 (d, 1, $J_{1,2}$ = 0 and $J_{1,8}$ = 3.5 Hz, H1), 4.67 (d, 1, $J_{1,2}$ = 0 and $J_{1,8}$ = 4 Hz, H2), 5.00 (d, 1, $J_{1,8}$ = 3.5 Hz, H8), 5.15, 5.40 (2t, 1 each, $J_{6,7}$ = 2 Hz, =CH_2), 5.93 (d, 1, $J_{2,6}$ = 4 Hz, H6).

*[1S-(1β,2α,6α,8β,9α)]-4,4,9-Trimethyl-3,5,7,11-tetraoxatricyclo[6.3.0.0^{2,6}]undecane (**70**)*

Catalytic hydrogenation of **69** (50 mg) over palladium-on-charcoal (10%, 50 mg) in ethanol (2.5 mL) for 2 h at 3.5 atm gave a mixture of **70** (88%) and **71** (12%) both with R_f 0.53 (*H*), olive-green color with anisaldehyde – sulfuric acid spray.

Nuclear magnetic resonance of **70** ($CDCl_3$) δ: 1.08 (d, 3, $J_{9,Me}$ = 7 Hz, 9-Me), 1.31, 1.47 (2s, 3 each 4-Me_2), 2.23 (m, 1, $J_{8,9}$ = 3.5, $J_{9,10a}$ = 7, and $J_{9,10b}$ = 10.5 Hz, H9), 3.35 (dd, 1, $J_{9,10b}$ = 10.5 and $J_{10a,10b}$ = 7.5 Hz, H10b), 3.90 (t, 1, *J* = 7.5 Hz, H10a), 4.43 (d, 1, $J_{1,2}$ = 0 and $J_{1,8}$ = 3.5 Hz, H1), 4.50 (d, 1, $J_{1,2}$ = 0 and $J_{2,6}$ = 3.5 Hz, H2), 4.68 (t, 1, $J_{1,8}$ = $J_{8,9}$ = 3.5 Hz, H8), 5.86 (d, 1, $J_{2,6}$ = 3.5 Hz, H6).

*[1S-(1β,2α,6α,8β,9β)]-4,4,9-Trimethyl-3,5,7,11-tetraoxatricyclo[6.3.0.0^{2,6}]undecane (**71**)*

Potassium azodicarboxylate (971 mg, 5 mmol) was added to a solution of **68** (198 mg, 1 mmol) in methanol (5 mL) and a solution of 2 *N* acetic acid in methanol (5 mL) was added to the yellow suspension at a rate of 1 mL/6 min under vigorous stirring. Stirring was continued for 1 h. Diethyl ether (10 mL) was added to the colorless mixture, the salts were filtered off, and the filtrate evaporated. The resulting residue was extracted with hot cyclohexane affording a mixture of **69** (35.9%), **70** (21.5%), and **71** (42.6%) as determined by glpc (column 3 m × 4 mm; OV-225, 10%, on GCQ 100/200; column temperature 80→190°C, 2°/min, retention times 54, 51.0, and 51.6 min, respectively).

Nuclear magnetic resonance of **71** ($CDCl_3$: 0.97 (d, 3, $J_{9,Me}$ = 7 Hz, 9-Me), 1.31, 1.47 (2s, 3 each, 4-Me_2), 2.42 (ddq, 1, $J_{9,10b}$ = $J_{9,Me}$ = 7 Hz and $J_{8,9}$ = 1 Hz, H9), 3.51 (dd, 1, $J_{9,10a}$ = 1.5 and $J_{10a,10b}$ = 8 Hz, H10a), 3.94 (dd, 1, $J_{9,10b}$ = 5.5 and $J_{10a,10b}$ = 8 Hz, H10b), 4.43 (d, 1, $J_{1,2}$ = 0 and $J_{1,8}$ = 3.5 Hz, H1), 4.54 (d, 1, $J_{1,2}$ = 0 and $J_{2,6}$ = 3.5 Hz, H2), 4.58 (dd, 1, $J_{1,8}$ = 3.5 and $J_{8,9}$ = 1 Hz, H8), 5.86 (d, 1, $J_{2,6}$ = 3.5 Hz, H6).

1. J. Berger. Ger. Patent No. 2,140,322 (February 17, 1972).
2. J. Berger. U.S. Patent No. 3,657,421 (April 18, 1972).
3. J. Berger, H. H. Lehr, S. Teitel, H. Maehr, and E. Grunberg. J. Antibiot. **26**, 15 (1973).
4. C. Vos and J. den Admirant. Ger. Patent No. 2,140,674 (May 18, 1972).
5. C. Vos and J. den Admirant. U.S. Patent No. 3,923,981 (December 2, 1975).
6. C. Vos and J. den Admirant. U.S. Patent No. 3,927,211 (December 16, 1975).
7. H. Wolf and H. Zähner. Arch. Mikrobiol. **83**, 147 (1972).
8. H. Maehr, M. Leach, L. Yarmchuk, and A. Stempel. J. Am. Chem. Soc. **95**, 8449 (1973).
9. R. Wax, W. Maiese, R. Weston, and J. Birnbaum. J. Antibiot. **29**, 670 (1976).
10. W. Maiese and R. Wax. U.S. Patent No. 4,024,251 (February 12, 1976).
11. H. M. Jongsma, H. J. Kooreman, J.L. Os, and C. Vos. Ger. Patent No. 2,621,615 (November 25, 1976).
12. I. Hummel. Diplomarbeit, Göttingen. 1977.
13. I. Thein. Dissertation, Tübingen. 1977.
14. S. B. Zimmerman, J. H. Chalmers, Jr., R. S. Dewey, and E. O. Stapley. J. Antibiot. **32**, 665 (1979).
15. M. W. Nimeck, E. Meyers, and W.-C. Liu. U.S. Patent No. 3,898,327 (August 5, 1975).

16. B. M. FROST, M. E. VALIANT, B. WEISSBERGER, and E. L. DULANEY. J. Antibiot. **29**, 1083 (1976).
17. M. W. STUTZ, S. L. JOHNSON, and F. R. JUDITH. J. Anim. Sci. **43**, 260 (1976).
18. H. WOLF, G. CHINALI, and A. PARMEGGIANI. Proc. Natl. Acad. Sci. U.S.A. **71**, 4910 (1974).
19. S. BROWN and T. BLUMENTHAL. J. Biol. Chem. **251**, 2749 (1976).
20. G. CHINALI, H. WOLF, and A. PARMEGGIANI. Eur. J. Biochem. **75**, 55 (1977).
21. H. WOLF, G. CHINALI, and A. PARMEGGIANI. Eur. J. Biochem. **75**, 67 (1977).
22. T. BLUMENTHAL and J. DOUGLASS. Abstr. Annu. Meet. Am. Soc. Microbiol. **77**, 194 (1977).
23. E. FISCHER, H. WOLF, K. HANTKE, and A. PARMEGGIANI. Proc. Natl. Acad. Sci. U.S.A. **74**, 4341 (1977).
24. A. PARMEGGIANI, E. FISCHER, O. FASANO, J. B. CRECHET, and H. WOLF. Chem. Abstr. **89**, 71471c (1978).
25. I. SMITH and P. PARESS. J. Bacteriol. **135**, 1107 (1978).
26. H. WOLF, D. ASSMANN, and E. FISCHER. Proc. Natl. Acad. Sci. U.S.A. **75**, 5324 (1978).
27. H. MAEHR, M. LEACH, L. YARMCHUK, and M. MITROVIC. J. Antibiot. **32**, 361 (1979).
28. W. L. MARUSICH, E. F. OGRINZ, and M. MITROVIC. Poult. Sci. **52**, 2059 (1973).
29. W. L. MARUSICH, E. F. OGRINZ, and M. MITROVIC. Poult. Sci. **53**, 936 (1974).
30. R. BEUKERS, J. G. OOSTENDORP, and C. J. VAN EEKEN. Abstracts, Second World Congress on Animal Feeding, Madrid. October 23–28, 1972. p. 127.
31. T. VAN EEK and R. BEUKERS. Ger. Patent No. 2,406,164 (August 14, 1974).
32. A. G. FOSTER and D. L. HARRIS. J. Anim. Sci. **43**, 252 (1976).
33. M. W. STUTZ, S. L. JOHNSON, K. G. OTTO, and B. M. MILLER. J. Anim. Sci. **43**, 259 (1976).
34. T. VAN EEK and R. BEUKERS. U.S. Patent No. 3,928,573 (December 23, 1975).
35. C.-M. LIU, T. HERMANN, and P. A. MILLER. J. Antibiot. **30**, 244 (1977).
36. J. UNOWSKY and D. C. HOPPE. J. Antibiot. **31**, 662 (1978).
37. H. MAEHR, J. F. BLOUNT, R. H. EVANS, JR., M. LEACH, J. W. WESTLEY, T. H. WILLIAMS, A. STEMPEL, and G. BÜCHI. Helv. Chim. Acta, **55**, 3051 (1972).
38. R. KUHN and T. WIELAND. Chem. Ber. **73**, 971 (1940).
39. R. BEUTEL and M. TISHLER. J. Am. Chem. Soc. **68**, 1463 (1946).
40. H. MAEHR, J. F. BLOUNT, M. LEACH, A. STEMPEL, and G. BÜCHI. Helv. Chim. Acta, **55**, 3054 (1972).
41. H. MAEHR, M. LEACH, T. H. WILLIAMS, W. BENZ, J. F. BLOUNT, and A. STEMPEL. J. Am. Chem. Soc. **95**, 8448 (1973).
42. H. MAEHR, J. F. BLOUNT, M. LEACH, and A. STEMPEL. Helv. Chim. Acta, **57**, 936 (1974).
43. A. F. THOMAS. J. Am. Chem. Soc. **91**, 3281 (1969).
44. G. HESSE and K. MIX. Chem. Ber. **92**, 2427 (1959).
45. H. MAEHR, T. H. WILLIAMS, M. LEACH, and A. STEMPEL. Helv. Chim. Acta, **57**, 212 (1974).
46. K. S. PFITZER and W. E. DONATH. J. Am. Chem. Soc. **81**, 3213 (1959).
47. R. TOLMAN, R. K. ROBINS, and L. B. TOWNSEND. J. Heterocycl. Chem. **4**, 230 (1967).
48. J. D. STEVENS and H. G. FLETCHER, JR. J. Org. Chem. **33**, 1799 (1968).
49. H. MAEHR, M. LEACH, J. F. BLOUNT, and A. STEMPEL. J. Am. Chem. Soc. **96**, 4034 (1974).
50. B. A. LEWIS, F. SMITH, and A. M. STEPHEN. Methods Carbohydr. Chem. **2**, 182 (1963).
51. W. C. BAIRD, J. B. FRANZUS, and J. H. SHRRIDGE. J. Am. Chem. Soc. **89**, 410 (1967).
52. H. OHURI, H. J. GORDON, J. G. MOFFATT, M. L. MADDOX, A. T. CHRISTENSEN, and S. K. BYRAM. J. Am. Chem. Soc. **97**, 4602 (1975).
53. C. VOS and P. E. J. VERWIEL. Tetrahedron Lett. 2823 (1973).
54. C. VOS and P. E. J. VERWIEL. Tetrahedron Lett. 5173 (1973).
55. C.-M. LIU, H. MAEHR, M. LEACH, M. LIU, and P. A. MILLER. J. Antibiot. **30**, 416 (1977).
56. C.-M. LIU, T. H. WILLIAMS, and R. G. PITCHER. J. Antibiot. **32**, 414 (1979).
57. W. C. HAMILTON. Acta Crystallogr. **18**, 506 (1965).
58. R. KUHN and H. TRISCHMANN. Chem. Ber. **96**, 284 (1963).
59. V. M. PARIKH and J. K. N. JONES. Can. J. Chem. **43**, 3452 (1965).