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# TETRACYCLIC TRITERPENOIDS FROM *MELIA AZEDARACH*, L.—III[1]

CHAO-KUO CHIANG[a] and F. C. CHANG*
University of Tennessee Medical Units, Memphis, Tennessee 38103

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**Abstract**—From petroleum ether extracts of the bark of *M. azedarach* four novel tetracyclic triterpenoids have been isolated and characterized to be $C_{30}$ compounds of the euphane (20*R*) series. Previously known triterpenoids from Meliaceae have all been 20*S* compounds. Kulinone **1** is the first known euphane or tirucallane (20*S*) derivative oxygenated in the D ring; kulactone **2**, kulolactone **3** and methyl kulonate **4** are additionally oxygenated in the side chain. Compounds **2** and **3** have a 2-oxa-*trans*-bicyclo[3,3,0]-octanone structure.

Recent chemical publications[2,3] on *Melia azedarach*, L. have been concerned primarily with components isolated from the fruit of the tree. The principal efforts have been by Lavie *et al.*[2] who described a number of novel triterpenes and have shown their possible relationship to the biogenesis of other compounds found in Meliaceae.

The tree bark has been used in Asiatic and African herb medicine[4,5] as an anthelmintic and a parasiticide. Early work was mainly concerned with the anthelmintic component, which was shown to be vanillic acid by Okahara and Taniguchi,[6] who also identified *dl*-catechol as a component of the bark extract. Nath[7] reported tannin and a substance "bakalactone ($C_{22}H_{20}O_4$)" from heartwood, but the latter has not been characterized. Recently Tsukamoto *et al.*[8] in a survey of drug plants for sterols and triterpenes by means of gas chromatography, identified (by retention times) campesterol, stigmasterol, sitosterol, β-amyrin, and lupeol in the bark of *M. azedarach* var. japonica. Ekong *et al.*[9] in a brief communication mentioned the presence of "cycloeucalenol and the corresponding 3-oxo compound, besides meliacins" in a sample of wood oil from *M. azedarach*, but gave no details.

This paper is an account of the isolation and characterization of novel tetracyclic triterpenes from petroleum ether extracts of tree bark obtained from Taiwan and Hong Kong.

By a combination of column and preparative TLC, with monitoring by GLC and TLC, four compounds were obtained. These were given the trivial names, kulinone **1**, kulactone **2**, kulolactone **3** and methyl kulonate **4**; the prefix of the names being derived from the romanized form of the Chinese name of the tree, "Kulien".

Kulinone **1**, colorless crystals out of light petroleum, proved to be the key intermediate in characterizing the group of compounds. Elemental analysis and high resolution mass spectrometry indicated the molecular formula $C_{30}H_{48}O_2$. Its IR spectrum showed OH (2·84 μ) and CO (5·84 μ) absorption to account for the two O atoms, in addition to double bond absorption at 6·0, 11·9 and 12·1 μ. Unsaturation was confirmed by its NMR spectrum which exhibited single olefin protons at τ4·88 and 4·7, and by positive response in a tetranitromethane test.

The NMR spectrum further displayed 18 upfield protons corresponding to six Me groups, and 6 protons at τ8·31–8·43, suggesting two vinyl-Me groups. Catalytic hydrogenation provided other clues to the structure of **1** and to the nature of the double bonds present. Different products are formed, (Fig 1) depending on whether the hydrogenation is carried out under neutral or under acidic conditions: (a) with methanol as solvent, with one mole of hydrogen absorption, a dihydrohydroxy ketone **1d** results, which on further hydrogenation is reduced to give **5d**, a diol. In the NMR spectra of **1d** and **5d**, both show a loss of one of the olefin resonances (τ4·88), as well as the original vinyl-methyl signals, the latter shifting upfield to the tertiary Me region. Compound **5d** resists further hydrogenation, but still reacts with tetranitromethane; (b) with acetic acid as hydrogenation solvent, a different diol **6d**, isomeric with **5d**, is formed. The compound gives a positive tetranitromethane reaction, although its NMR spectrum shows no olefin resonance; evidently a double bond has shifted into a tetrasubstituted position.

These observations on molecular weight and formula, IR and NMR spectra, the presence of two double bonds one of which can shift from a trisubstituted to a tetrasubstituted position, were strongly suggestive that **1** is a compound of $\Delta^{7,24}$-tetracyclic triterpene type[10a]-(such as butyro-

[a]In part excerpted from the Ph.D. dissertation of Chao-kuo Chiang, Department of Pharmacognosy, 1969.

*To whom pertinent correspondence should be addressed.

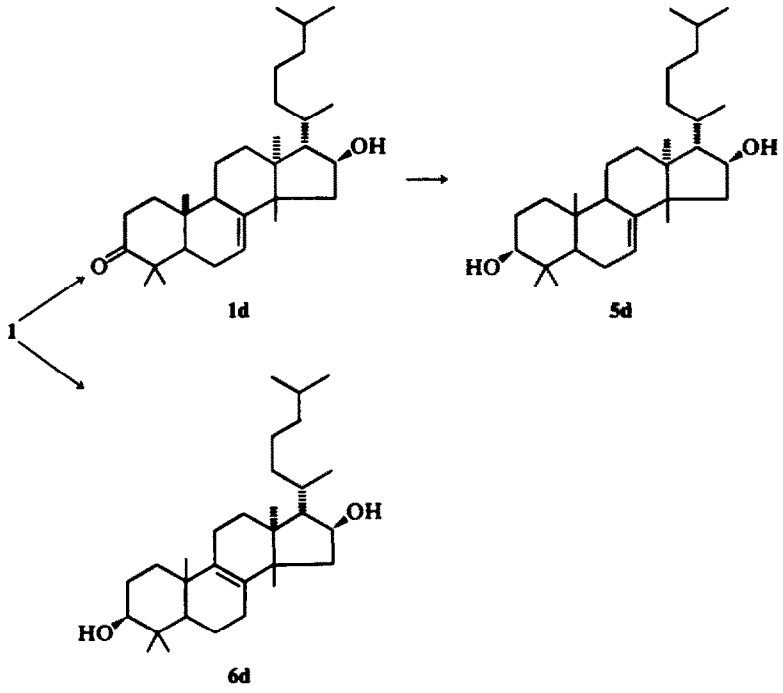



Fig 1.

spermol, $\alpha$-elemonic acid, masticadienonic acid), with one keto and one hydroxyl substituent. The OH group was readily acetylated at room temperature, hence is not tertiary. Since a triterpene without an oxygen substituent at C-3 is rare, kulinone was considered to be either a 3-oxo or 3-ol derivative, with the former more probable for several reasons: (a) in the NMR spectrum the signal for a proton geminal to OH did not resemble those (either 3$\alpha$ or 3$\beta$) in known 4,4-dimethyl triterpenes, (b) the 3-oxo assignment fits the CO absorption value (5·84 $\mu$), and is consistent with the easy formation of an ethylene ketal derivative **1c**, and (c) most significant of all, the optical rotatory dispersion curve of **1** with its negative Cotton effect, $[\phi]_{321}$ −1,370°, is in agreement with the values found for a number of recently characterized $\Delta^7$-3-oxo triterpenoids from Meliaceae or closely related families (flindissone lactone,[10b] $[\phi]_{315}$ −2,650°; bourjotinolone A,[11] $[\phi]_{314}$ −1,350°; bourjotinolone B,[11] $[\phi]_{310}$ −2,740°; melianone,[2a] $[\phi]_{315}$ −2,250°; melianodiol,[2b] $[\phi]_{315}$ −1,483°).

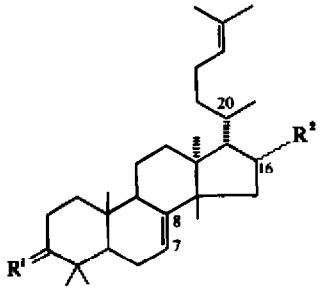
We surmised that the parent triterpene of kulinone was tirucallane (13$\alpha$,14$\beta$,20$\alpha$-H) because all known triterpenes from Meliaceae had been such derivatives. To test this speculation the methanesulfonate derivative **1b** of kulinone was reduced with LAH, resulting in a mono-hydroxy compound $C_{30}H_{50}O$, which when hydrogenated should be $\Delta^7$-tirucallen-3$\beta$-ol **7**,[10c] a known compound. Attempts to locate a sample of **7** or of its more common $\Delta^{8,24}$-relative tirucallol **8**,[12] were unsuccessful, but fortuitously our admittedly less than optimum choices of comparison compounds butyrospermol[13] and euphenol[14] turned out to be the appropriate ones. The compound was found to be identical with butyrospermol **9** and its hydrogenation product in acetic acid accordingly[14b] was identical with euphenol **10**. (Fig 2).

Correlation of kulinone with butyrospermol establishes that the former is a 3-oxo-$\Delta^{7,24}$-euphadiene ( a 20$\beta$-H or 20R derivative) with only the position and configuration of the additional OH group lacking for a complete structure. The fact that the OH group is secondary (see below), and not in an allylic position (as shown by NMR), limits the number of possible positions. The diketone **11** obtained by Jones' oxidation of kulinone proved to be the key intermediate in the characterization of the OH group. The diketone **11** has its new CO group IR absorption at 5·73 $\mu$, in the cyclopentanone region,[15] and the ORD curve of its 3-ketal derivative **11c** has a strong positive Cotton effect ($[\phi]_{333m\mu}$ +11,300°), characteristic of 16-keto compounds of the 2-oxo-A-norsteroid type of *trans*-hexahydroindan-2-ones.[16]

Reduction of the 3-ketal **11c** by LAH yields epimeric alcohols **1c** and **12c**; each alcohol can be re-oxidized to the original ketal **11c** and the minor one is identical with the ketal **1c** of kulinone (Fig 3). Assignment of configuration of the alcohols was made on the basis of three types of evidence:

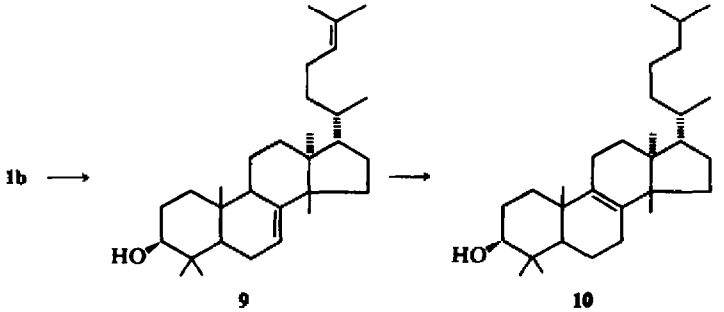
(a) The ratio of the epimers was 3:2. In metal hydride reductions,[17] hydride attack is favored from the less hindered direction at the site of reaction, which as molecular models show, is the β-side of the D-ring in **11c**, and the α-ol would be expected to be the major reduction product.

(b) Table 1 shows the molecular rotation differences associated with kulinone, 16-epikulinone **12**, their acetates **1a** and **12a**, and the unsubstituted (at C-16) compound butyrospermone **13**. For kulinone ΔOH and $\Delta_1$ are both positive, and for the epi compound both are negative. According to




| | $R^1$ | $R^2$ | |
|---|---|---|---|
| **1** | O | β—OH | |
| **1a** | O | β—OAc | |
| **1b** | O | β—$OSO_2Me$ | |
| **1c** | —$OCH_2CH_2O$— | β—OH | |
| **1d** | O | β—OH | 24,25-dihydro |
| **5** | H,β—OH | β—OH | |
| **5a** | H,β—OAc | β—OAc | |
| **5d** | H,β—OH | β—OH | 24,25-dihydro |
| **6a** | H,β—OAc | β—OAc | $\Delta^8$; 24,25-dihydro |
| **6d** | H,β—OH | β—OH | $\Delta^8$; 24,25-dihydro |
| **7** | H,β—OH | H | 20S; 24,25-dihydro |
| **8** | H,β—OH | H | $\Delta^8$; 20S |
| **9** | H,β—OH | H | |
| **9a** | H,β—OAc | H | 24,25-dihydro |
| **10** | H,β—OH | H | $\Delta^8$; 24,25-dihydro |
| **11** | O | O | |
| **11c** | —$OCH_2CH_2O$— | O | |
| **12** | O | α—OH | |
| **12a** | O | α—OAc | |
| **12c** | —$OCH_2CH_2O$— | α—OH | |
| **13** | O | H | |
| **27** | H,H | H,H | |

All are 20R(20β),$\Delta^{7,24}$-compounds, except where indicated.




**9** **10**

Fig 2.

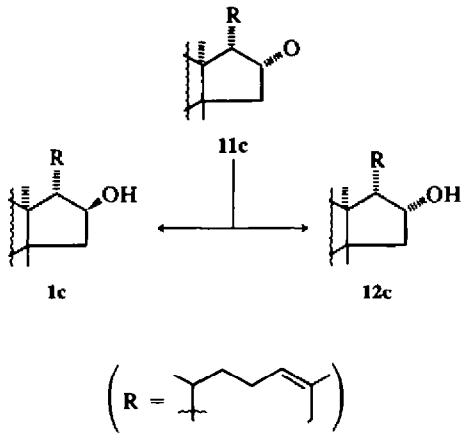



Fig 3.

kulinone has an analogously large effect (14 Hz) on the 13-Me and a small effect (2 Hz) on the 14-Me group.

On the basis of these criteria a tentative assignment was made of 16$\beta$ to the OH group, which became secure only when the structure of kulactone (see below) was elucidated. Kulinone **1** is thus 16$\beta$-hydroxyeupha-7,24-dien-3-one (IUPAC: 16$\beta$-hydroxy-5$\alpha$,13$\alpha$,14$\beta$,17$\alpha$-lanosta-7,24-dien-3-one) or 16$\beta$-hydroxybutyrospermone.

Kulactone **2**, crystallized from the more polar chromatographic fractions after removal of kulinone, resembled kulinone in a number of spectral characteristics. Its IR spectrum also had alkene bands at 6·0, 11·9 and 12·1 $\mu$ and CO absorption at 5·83 $\mu$, but had an additional band in the $\gamma$-lactone CO region at 5·59 $\mu$. Its NMR spectrum exhibited the $\tau$4·63 and 4·88 olefinic signals, the two vinylmethyl signals at $\tau$8·36 and 8·30, and a 1-proton multiplet at $\tau$5·83, analogous to those in kulinone; but in the high field region, 15 protons (5 sharp singlets) were seen instead of the 18 of kulinone.

Table 1. Molecular rotation differences involving butyrospermone **13**, kulinone **1** and 16-epikulinone **12**, and the acetates, **1c** and **12c**

| | $16\text{-}H_2$[a] | $[M]_D$ 16-OH | 16-OAc | $\Delta[M]_D$ $\Delta$OH | $\Delta$OAc | $\Delta_1$($\Delta$OAc-$\Delta$OH) |
|---|---|---|---|---|---|---|
| Kulinone (16$\beta$) | −170° | −88° | −24° | +82° | +146° | +64° |
| Epikulinone (16$\alpha$) | −170° | −207° | −434° | −37° | −264° | −227° |

[a]Value of butyrospermone **13** taken from literature.[13]

Klyne and Stokes,[18] kulinone would be the 16$\beta$ compound.*

(c) In the NMR, the 13$\alpha$ and 14$\beta$ Me protons should be deshielded unequally by a OH substituent at C-16, with the extent of deshielding dependent on the configuration of the latter. A 16$\alpha$ group would be expected to have a greater deshielding effect on the 13$\alpha$ Me group, whereas a 16$\beta$ group should have a greater effect on the 14$\beta$ Me.[19]

Accordingly, for kulinone chemical shifts† of the 13$\alpha$-Me and 14$\beta$-Me groups are at 50 and 76 Hz, respectively, while with epikulinone both are at 62 Hz. When compared with the corresponding signals in butyrospermone **13** ($H_2$ at C-16) of 48 and 60 Hz, the 16$\beta$-OH group of kulinone has a 16 Hz deshielding effect on the 14-Me and a 2 Hz effect on the 13-Me, whereas the 16$\alpha$-OH of epi-

Like kulinone **1**, under neutral conditions one double bond of kulactone was catalytically hydrogenated readily, to give **2d**, and the second was resistant to further hydrogenation, and also like **1** a second mol of hydrogen reduces the keto group to an alcohol, **14d**.

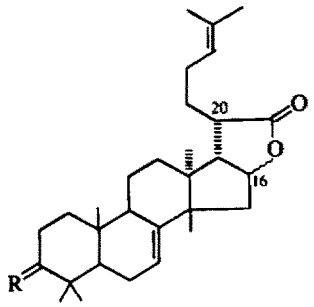
The many points of resemblance between kulactone and kulinone suggested that the two compounds are closely related, and that a lactone moiety, indicated by the IR spectrum of kulactone, would be a key to revealing the difference between the two compounds.

At room temperature kulactone on treatment with sodium borohydride in methanol gives two products, **14** and **25**. Compound **14**, according to its IR spectrum, has the lactone grouping intact but the keto group has been reduced to hydroxy, and **25** is evidently a cleavage product of **14**, yielding a diol methyl ester.

Kulactone is cleaved by either methanolic hydrochloric acid or methanolic potassium hydroxide to yield the same methyl hydroxy ester **4**, consistent with the presence of a lactone grouping. This methyl ester **4**, which subsequently was isolated

*However, exceptions have been noted [(e.g., W. O. Godtfredsen, W. V. Daehne, S. Vangedal, A. Marquet, D. Arigoni, and A. Melera, *Tetrahedron* 3505 (1965)].

†The chemical shifts, given in Hz (60 MHz) from TMS, are assigned by correlating the methyl signals observed in the various compounds obtained in this work, supplemented and supported by the assignments derived by D. Lavie, Y. Shvo and E. Glotter [*Tetrahedron* **19**, 2255 (1963)] from a series of euphane and isoeuphane derivatives. A detailed NMR analysis of our compounds will be communicated separately.

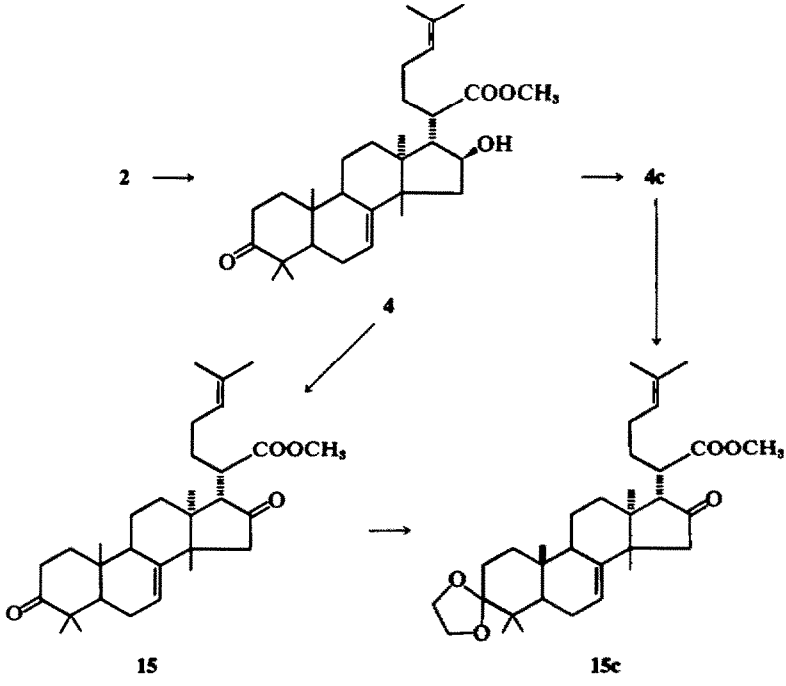



| | R | α or β at C-16 | |
|---|---|---|---|
| 2 | O | β | |
| 2c | —$OCH_2CH_2O$— | β | |
| 2d | O | β | 24,25-dihydro |
| 3 | H,α—OH | β | |
| 3a | H,α—OAc | β | |
| 14 | H,β—OH | β | |
| 14d | H,β—OH | β | 24,25-dihydro |
| 17 | O | α | |
| 17c | —$OCH_2CH_2O$— | α | |
| 17d | O | α | 24,25-dihydro |
| 26 | H,β—OH | α | |

All are 20R(20β),$\Delta^{7,24}$-compounds, except where indicated.

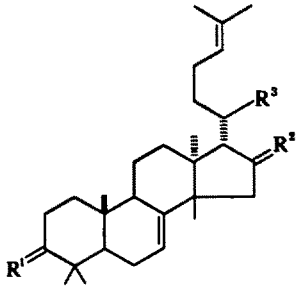
directly from the plant extracts, (see below) and was given the name methyl kulonate (acid: kulonic), forms an acetate **4a**, a mesylate **4b**, and ethylene ketal **4c**. It (**4**) was oxidized to a dehydro compound **15**, a diketo methyl ester, which forms a monoketal **15c** identical with the oxidation product of the ketal **4c** of methyl kulonate (Fig 4). The new keto groups of both **15** and **15c** were shown by IR and ORD measurements to be at C-16, in the same way as described above for the diketone **11** prepared from kulinone.

Kulactone was successfully correlated with




15 15c

Fig 4.



| | $R^1$ | $R^2$ | $R^3$ | |
|---|---|---|---|---|
| **4** | O | H,β—OH | —COOMe | |
| **4a** | O | H,β—OAc | —COOMe | |
| **4b** | O | H,β—OMs | —COOMe | |
| **4c** | —$OCH_2CH_2O$— | H,β—OH | —COOMe | |
| **4d** | O | H,β—OH | —COOMe | 24,25-dihydro |
| **4e** | 2,4-dinitro-phenylhydrazone | H,β—OH | —COOMe | |
| **15** | O | O | —COOMe | |
| **15c** | —$OCH_2CH_2O$— | O | —COOMe | |
| **16** | O | H,β—OH | —$CH_2OH$ | |
| **16a** | O | H,β—OAc | —$CH_2OAc$ | |
| **16c** | —$OCH_2CH_2O$— | H,β—OH | —$CH_2OH$ | |
| **16e** | —$OCH_2CH_2O$— | H,β—OH | —$CH_2OTr$ | |
| **16f** | —$OCH_2CH_2O$— | H,β—OAc | —$CH_2OAc$ | |
| **18** | O | H,α—OH | —$CH_2OH$ | |
| **18a** | O | H,α—OAc | —$CH_2OAc$ | |
| **18c** | —$OCH_2CH_2O$— | H,α—OH | —$CH_2OH$ | |
| **18e** | —$OCH_2CH_2O$— | H,α—OH | —$CH_2OTr$ | |
| **19** | —$OCH_2CH_2O$— | O | —$CH_2OTr$ | |
| **23** | O | H,β—OH | —COOH | |
| **25** | H,β—OH | H,β—OH | —COOMe | |
| **25a** | H,β—OAc | H,β—OAc | —COOMe | |
| **28** | H,α—OH | H,H | —COOMe (20S) | |

All compounds are 20R with the exception of **28**; Tr = trityl group.

kulinone through methyl kulonate by the reactions indicated in Fig 5. Methyl kulonate ketal **4c** on reduction with LAH gave rise to a diol ketal **16c**, which was selectively mesylated. The mesylation product without isolation of the components was treated with LAH. The product consisted of unchanged starting diol ketal **16c**, and a compound which had a NMR spectrum identical with that of kulinone ketal **1c**, and which on acid hydrolysis afforded kulinone.

Kulactone ketal **2c** on reduction with LAH yields the aforementioned diol ketal **16c**, derived from methyl kulonate, and in so doing establishes the fact that neither cleavage of the lactone by acid nor by base affects the configurations at C-16, 17 or 20; and consequently the oxido linkage of the lactone is at C-16 and has the same configuration as kulinone at that position. Since kulactone is a γ-lactone, as indicated by the high frequency of its CO absorption, the carbon point of attachment of the lactone CO is limited to positions 13, 14 and 20; this being dictated by the structure of the parent hydrocarbon, now known to be eupha-7,24-diene **27**.

It is unlikely that the carbomethoxy group of methyl kulonate **4** is at either of the angular positions, 13 and 14, as alkaline hydrolysis studies, carried out on **4** and its corresponding 16-oxo methyl ester **15**, show that hydrolysis proceeds too fast for a tertiary carbomethoxy group, even one activated by a γ-situated keto or OH group,[20] and furthermore the mass spectrum* of **4** does not show the M-COOMe fragment expected for a tertiary carboxylic acid ester.[21] In addition, with the oxido linkage of kulactone at 16, one of the two positions, 13 or 14, for the carboxyl group would be untenable, a 5-membered *trans*-lactone would be an excessively strained structure. The strongest evidence for placing the lactone CO at C-20 is found in the NMR spectra of both kulactone and methyl kulonate, in which five clean sharp Me signals are present; a Me group at C-20 would be

*An analysis of the mass spectra of some of the compounds reported here will be communicated separately.

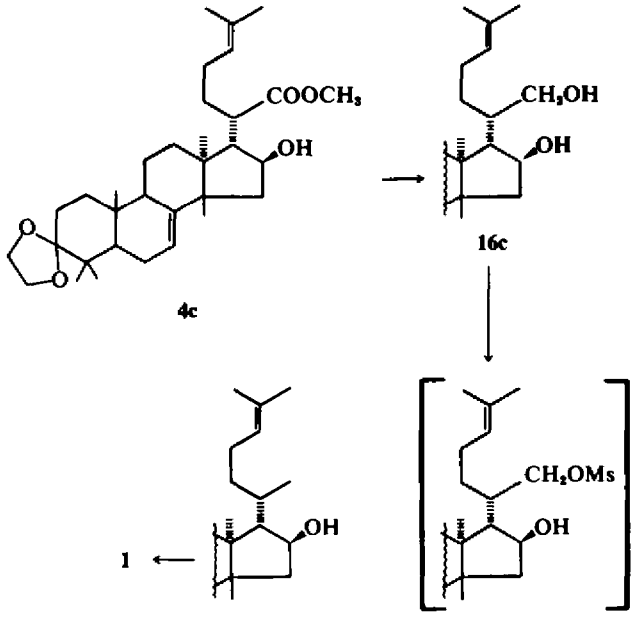



Fig 5.

secondary and a split Me signal would be expected, as is found for kulinone.

The evidence that kulactone and kulinone have the same configuration at C-16, and that the CO of the γ-lactone group of kulactone is attached to C-20, combined with the known α-side chain orientation of euphanes, initially appeared to require a reassignment of the 16-OH configuration of kulinone. The β-configuration would mean that the lactone is a γ-lactone *trans*-fused to a 5-membered ring, a 2-oxa-*trans*-bicyclo[3,3,0]-octanone structure (Fig 6), regarded to be too highly strained to be stable;[22a] only *cis*-fused examples of the structure were known.[22b] In fact several futile attempts to prepare a compound of this type are in the recorded literature.[23] Therefore, we were inclined to believe that the assignment of 16β-ol to kulinone was erroneous in spite of the circumstantial evidence previously cited favoring the assignment, in addition to some further indications that the kulactone was not a typical γ-lactone: IR CO absorption frequency was somewhat high for an ordinary γ-lactone; the lactone group was readily cleaved by mild acid treatment, and the cleaved product resists re-lactonization.

However, subsequent experimentation shows that the original characterization of kulinone is

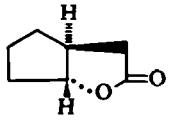



Fig 6.

correct, a kulactone is indeed a *trans*-γ-lactone: methyl kulonate mesylate **4b** when refluxed with collidine (to effect an elimination reaction) unexpectedly yielded a product **17** which was not a methyl ester (by NMR) but which did have a CO in the lactone region at a frequency slightly lower than that of kulactone. The lactone group of the new compound does not involve the 24,25-double bond, as dihydro methyl kulonate **4d** undergoes a similar reaction to give a dihydro lactone **17d**. The new lactone **17** was later found to be formed in 70% yield when without isolation the mesylation reaction product was refluxed, (Fig 7).

Lactone **17** was obtained in another reaction: When methyl 16-dehydrokulonate 3-ketal **15c** was reduced with $NaBH_4$, as expected, two products resulted, but the two products instead of being the epimeric pair of C-16 alcohols, consisted of one alcohol, methyl kulonate ketal **4c**, and a compound which was not an alcohol but was the ketal of the new lactone **17** (Fig 7). The new lactone is isomeric with kulactone and has quite similar spectroscopic properties, the chief differences being the slightly lower frequency (1773 $cm^{-1}$) of the lactone band in the IR spectra, and the chemical shift, band width and splitting pattern of the signal assigned to the C-16 proton in the NMR. The relationship of kulactone to compound **17** was revealed by reactions shown in Fig 8: 16-Epikulactone **17**, in the form of its 3-ketal **17c**, was reduced by LAH to a diol **18c**, isomeric with **16c** produced analogously from kulactone. The two diol ketals each reacted with trityl bromide to form a different primary monotrityl ether,[24] **16e** and **18e**. However, when

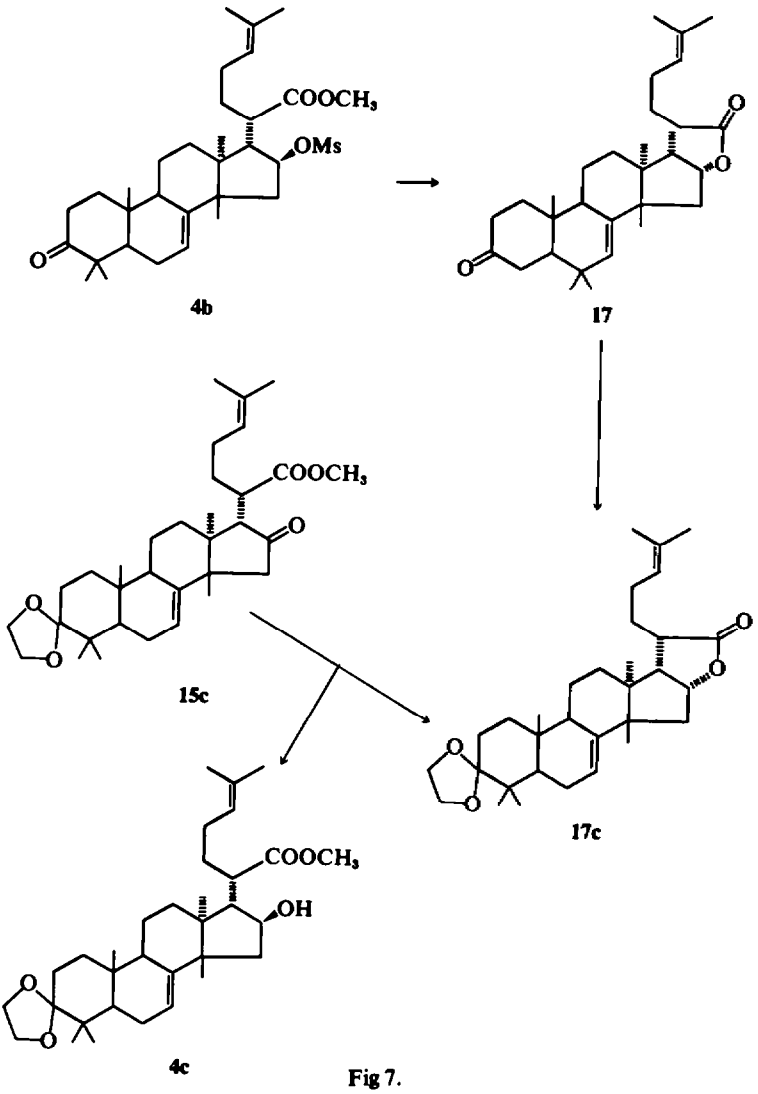



Fig 7.

oxidized they yielded the same keto compound **19**, affording evidence for the epimeric relationship of the two C-16 diols **16c** and **18c**, and therefore of kulactone and epikulactone.

The assignments of kulactone (*trans*) and epi compound (*cis*) are clear from the experimental evidence, especially the reaction described above in which 16-epikulactone ketal **15c** is formed in the $NaBH_4$ reduction of the 16-oxo compound **15c**. Apparently both hydroxy epimers at C-16 are formed initially, but the *cis*-hydroxy ester lactonizes spontaneously, accounting for the absence of the 16α-ol derivative in the reduction mixture.

An analogous finding was encountered by Hückel and Gelmoth[23a] who found that either by catalytic hydrogenation or sodium amalgam reduction, cyclopentanone-2-acetic acid methyl ester **20** gave a *cis*-fused lactone **21**, and a *trans*-hydroxy acid **22**, (Fig 9). Establishment of the *trans*-configuration of kulactone therefore confirms the 16β-OH configuration of kulinone.

Additional support that 16-epikulactone is the *cis*-isomer comes from an attempt to selectively mesylate diol **18** (derived from **17**), analogous to the first step in the conversion of kulactone to kulinone. No mesylate was formed; instead a compound characterized as 16α-21-epoxyeupha-7,24-dien-3-one **24** was obtained (Fig 10). Ring closure would be expected to take place with such facility only for the *cis*-isomer.[23c]

Thus kulactone has been characterized* to be

*This structure subsequently has been confirmed by X-ray crystallographic determination of 24,25-dibromokulactone [K. W. Ma, F. C. Chang and J. C. Clardy, *Chem. Comm.* 424 (1971)].

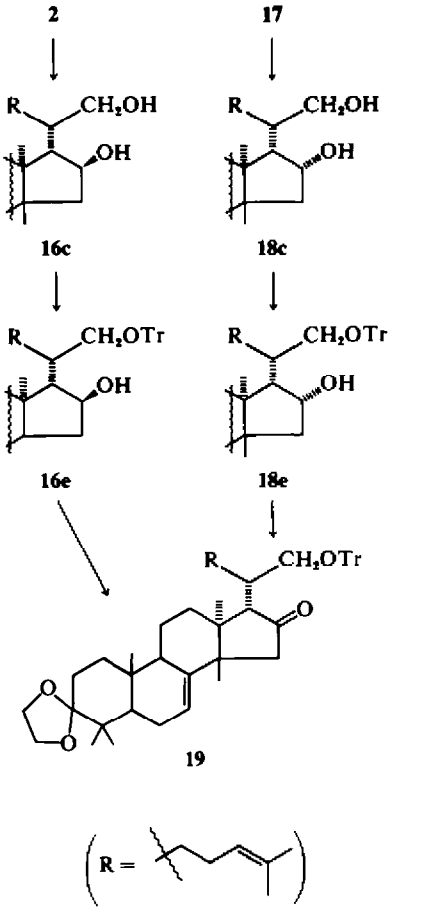



Fig 8.

16β-hydroxy-3-oxoeupha-7,24-dien-21-oic acid 21 → 16 lactone (IUPAC: 16β-hydroxy-3-oxo-5α, 13α,14β,17α-lanosta-7,24-dien-21-oic acid 21 → 16 lactone).

*Kulolactone* **3**, isolated from the mother liquors of the kulactone crystallization, and purified by preparative layer chromatography, was amorphous but homogeneous by TLC and GLC, and formed a crystalline acetate **3a**. Compound **3**, has an IR spectrum very similar to that of the $NaBH_4$ reduction product* **14** of kulactone; both compounds had the lactone CO but not the cyclohexanone band.

*In this reduction of kulactone by $NaBH_4$ no more than traces of the 3α (axial) alcohol were observed (TLC); only the 3β (equatorial) product was isolated, as compared with a 15% yield of the 3α-ol obtained when methyl 3-oxotirucalla-8,24-dien-21-oate **28** was reduced.[25a] Similarly every 3-keto compound in this work which was reduced with $NaBH_4$ gave the same results, virtually completely stereospecific reaction to the equatorial alcohol, as was also true when the same ketones were hydrogenated under neutral conditions with $PtO_2$ as catalyst.

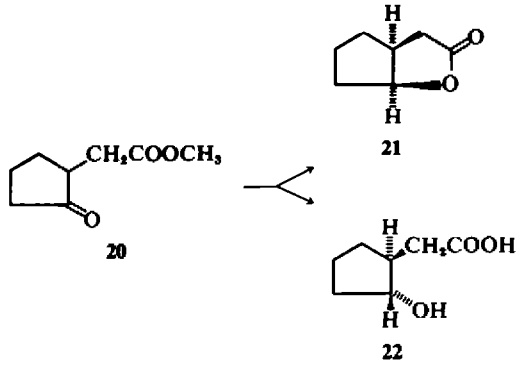



Fig 9.

In the NMR, the chief difference was in the signal due to the proton geminal to OH. The two compounds, **3** and **14**, are epimeric C-3 alcohols; both on oxidation yield kulactone. Comparison of the NMR CHOH signals of the two alcohols identifies kulolactone as the 3α-ol derivative.[25] This is compatible with the observation that although the less stable 3α-ols are rare among other triterpenoid classes, 3-hydroxy tetracyclic triterpenes from Meliaceae normally have the 3α(axial) orientation.[25]

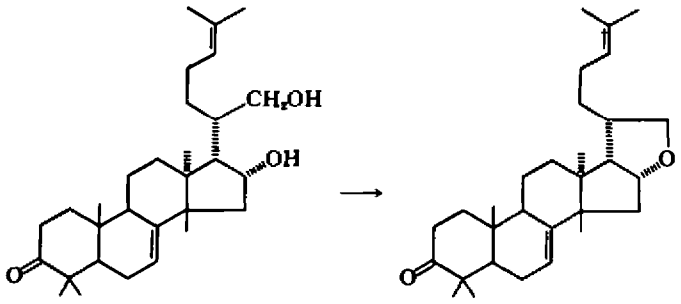
*Methyl kulonate* **4**. Recognition that this cleavage product was the likely biogenetic precursor to kulactone, prompted a search for the former compound in the plant extract. The successful efforts to identify and isolate the compound were of course simplified by having the cleavage product in hand. Since previously we had found that methyl kulonate was formed during prolonged recrystallization of kulactone with methanol as solvent, it was necessary to insure that the ester did not arise by cleavage of kulactone in the course of the extraction process, by excluding methanol or other possible methylating agents as solvents.

*Kulonic acid* **23**, was obtained by hydrolysis of kulactone in aqueous potassium hydroxide solution. Re-esterification with diazomethane gave methyl kulonate.

Attempts to lactonize kulonic acid by using several recently introduced reagents for preparing lactones (dicyclohexylcarbodiimide under reflux,[27] dicyclohexylcarbodiimide at room temperature,[28] *p*-toluene sulfonic acid[28]) were failures.

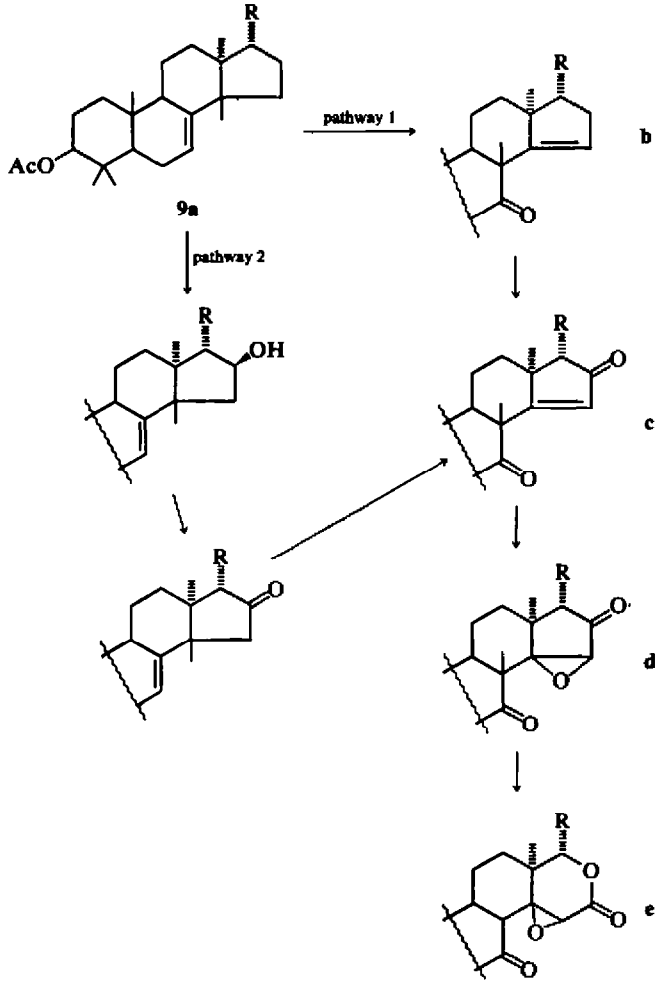
## DISCUSSION

The group of compounds from *M. azedarach* constitutes the first members of the euphane (20*R*) series to be reported from Meliaceae plants, and also the first compounds of the 13α,14β-tetracyclic triterpenes (euphane or tirucallane) class to have an oxygen substituent in the D-ring. However, a number of D-ring oxygen-substituted compounds in the lanostane (13β,14α) class are known, such as polyporenic acid[29] (15-OH), sulfurenic acid[30]




18

Fig 10.



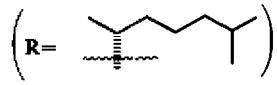




Fig 11.

(15-OH), tumulosic acid[31] (16-OH), all of which are products of wood rotting fungi.*

In 1960 Arigoni, Barton, Corey and Jeger, and their respective colleagues collaborated on a publication[32] elucidating the structure of the $C_{26}$ compound limonin, and suggested a biogenetic scheme for the formation of limonin from a tetracyclic triterpenoid precursor, such as butyrospermol. That part of the proposed pathway (Fig 11, pathway 1) pertinent to our discussion involving changes only in rings B, C, and D, was supported by a known chemical reaction[33] in which dihydrobutyrospermol acetate **9a** when oxidized yielded a compound with the partial structure represented by **b**, and all other steps represent known chemical reactions.

Subsequently, because of heightened interest in the biogenesis of a large number of complex compounds (now called meliacins[34]), related to limonin which had been isolated mainly from the two plant families Meliaceae and Rutaceae, much supporting evidence for the basic scheme of Arigoni *et al.*, has become available. The evidence consists of two types: First, compounds with structures representing postulated intermediates have been isolated, some from the same plant source, and secondly, successful conversions of the types in the proposed scheme have been carried out chemically.

*Isolation evidence.* From *Azadirachta indica* (fruits) four compounds were isolated[35] which had in rings B, C, and D the structures b, c, d, and e of the scheme (with acetoxy in place of ketone at C-7), and from other Meliaceae a wide assortment of $C_{26}$ and $C_{30}$ compounds representing various stages of oxidation and degradation, which lend support to the basic biogenetic scheme, have been reported from a number of laboratories.[34, 37, 39, 40]

From *M. azedarach* the number of known analogous compounds has been much more limited. The only compounds of possible significance associated with this postulated scheme were the $C_{30}$ compounds related to melianone, all reported by the Lavie group,[2] and the only known compounds of the meliacin type were nimbolins A and B described by Ekong *et al.*[38]

The compounds reported in the present work extend the range and types of compounds of possible intermediate stages of oxidation. They might be involved in an alternate biogenetic route (Fig 11, pathway 2) in which the initial triterpenoid precursor, such as butyrospermol, could undergo hydroxylation at 16 to give kulinone, which, either in its OH or keto form, might oxidatively rearrange probably through the 7,8-epoxide, to the equivalent of intermediate **c**. The presence of kulactone and methyl kulonate suggests that oxygenation of the side chain could precede the rearrangement process in the ring system. At present there is no evidence for the order in which the oxidative steps take place; whether the side chain changes take place before or after those in the ring, is unknown. Perhaps among different plant species the order of the steps, and even the overall biogenetic pathway as well, may vary.

*Chemical evidence.* Halsall *et al.*, have published processes[36] for the chemical conversion of methyl $\Delta^7$-elemolate derivatives to compounds of the meliacin type in the ring system, and also for the degradation of turraeanthin derivatives to simple meliacins.

Lavie *et al.*[2d] have succeeded in interconverting by stepwise oxidation the four meliacins (mentioned above) that they had isolated previously from *Azadirachta indica*.

It must be emphasized that the accumulated evidence in support of the proposed scheme, although substantial and increasing, is entirely of a circumstantial nature, and the scheme must be considered to be still speculative, until direct evidence, such as might come from biogenetic studies with isotopically labelled compounds, is obtained.

## EXPERIMENTAL

Microanalyses were performed by Weiler and Strauss, Oxford, England, and Galbraith Laboratories, Knoxville, Tennessee. M.ps were determined on an electrical micro hot stage and are uncorrected. Optical rotations were measured for solns in chloroform at room temp, 23–25°, with a Carl Zeiss Photoelectric Precision Polarimeter 0·005°. ORD and CD spectra were recorded on a Cary 60 spectropolarimeter at room temp. IR spectra were obtained on a Perkin-Elmer Infracord No. 137. Mass spectra were obtained on various instruments: JMS-01S (Jeolco), MS902, and M66 (Varian).

NMR spectra were taken for solns in $CDCl_3$ with TMS as internal standard with a Varian A-60A spectrometer; the exceptions are several in which a HA-100 was used. Chemical shifts are recorded in $\tau$ units, followed by a description of the signals in abbreviated form. Abbreviations: b = broad, s = singlet, d = doublet, t = triplet, m = multiplet; the Arabic numeral denotes the number of protons in signal; $w/2$ = width at half height and $J$ = coupling constant, both given in Hz; type of proton is indicated by underline unless it is self-explanatory; signals due to same type of proton are grouped together followed by a common description, where the number of protons represents the total present in all signals; and where a doublet is shown as d°, it is actually not a first-order doublet, although chemical shift is taken at the center and $J$ is the difference in Hz between the doublet.

Light petroleum used was Skelly-solve B (Skelly Oil Co.), b.p. 63–70°, further purified by $H_2SO_4$ treatment and distillation. Florisil for column chromatography was 60–100 mesh, product of Floridin Company.

For analytical TLC 0·25 mm thick layers of silica gel H (Merck Co. Darmstadt) on glass plates were used. After development, spots were visualized by spraying with EtOH-$H_2SO_4$-vanillin.[41] For preparative TLC plates

*Subsequent to our preliminary report[1] a publication[39] described the isolation of a lanostane derivative oxygenated at C-16, from Swietenia mahagoni (Meliaceae).

were layered 1·0 mm thick with silica gel H impregnated with "Ultraphor" (Badische Anilin-und Soda-Fabrik), and viewed under long wavelength UV light. Analytical plates were air-dried only, while preparative plates were in addition heated at 110° for 1 hr. The following solvent systems were employed for development: AB85 indicates light petroleum-EtOAc (85:15, by vol); AB80, (80:20); AB50 (50:50); and ABJ (50:48), light petroleum-EtOAc-AcOH (50:48:2). For preparative plates, 2X, 3X, etc. indicate that plates were multiply developed, 2, 3, etc. times.

*General procedures for preparation of derivatives*

Where a general procedure is used, details of the preparation, including processing of product, are described fully only in the first such preparation, i.e., kulinone acetate **1a**, kulinone mesylate **1b**; kulinone 3-ethylene ketal, **1c**; only in the instances where departure from the general procedure was required are details included in the other experimental descriptions.

*Isolation of triterpenes*

The air-dried bark (1100 g) of *Melia azedarach*, L. (Taiwan) ground to a powder was extracted with 5 liters of light petroleum in a 12-1. Soxhlet extractor for 24 hr. The hot soln after filtration was concentrated to one liter and refrigerated. A yellowish ppt (A, 3·9 g) was removed and the filtrate was evaporated to give a dark green residue (B, 14 g).

The ppt A appeared to be a complex mixture of long chain alaphatic compounds and was not investigated further in this work. The residue B was the source of the compounds described here, and a typical isolation experiment is described:

Five grams of B was dissolved in light petroleum and chromatographed on a 100 g column of Florisil. Fractions were taken as follows:

| Fraction | Eluting solvent light pet.-ether | Volume | Wt. of Residue, mg |
|---|---|---|---|
| 1 | 20:1 | 500 | 170 |
| 2 | 15:1 | 500 | 311 |
| 3 | 10:1 | 2000 | 572 |
| 4 | 5:1 | 1000 | 300 |
| 5 | 3:1 | 1500 | 386 |
| 6 | 1:1 | 1000 | 470 |
| 7 | ether | 2000 | 500 |

The residue from fraction 3, 572 mg, when dissolved in methanol and concentrated, yielded a crystalline solid (120 mg) which by TLC and GLC analyses, and IR and NMR comparisons, appears to be mainly sitosterol. The mother liquor by preparative TLC (AB80, 3X) afforded three fractions, listed according to decreasing $R_f$ value: (a) a complex mixture (115 mg), (b) kulinone (**1**, 138 mg, 2·8%*), and (c) sitosterol (118 mg).

The residue from fraction 5, 386 mg, when crystallized from MeOH gave crude crystals of kulactone **2**. The mother liquor by preparative TLC (AB80, 3X) yielded more kulactone, and kulolactone **3**. Total yield of kulactone was 290 mg (5·8%) and of kulolactone was 80 mg (1·6%).

The residue from fraction, 6, 470 mg, by preparative TLC (AB80, 4X), yielded 255 mg (5·1%) of methyl kulonate.

Other fractions of the column chromatography are not included in this report. More polar material eluted by methanol solvent mixtures also are not discussed.

*Kulinone* (**1**) was recrystallized from light petroleum as colorless prisms; m.p. 137·0–138·0°; $[\alpha]_D$ −20° (*c*, 1·2); ORD (*c*, 0·15; MeOH): $[\phi]_{600}$ −74°; $[\phi]_{589}$ −74°; $[\phi]_{321}$ −1,370°; $[\phi]_{270}$ +980°; $[\phi]_{254}$ +920°; $[\phi]_{240}$ +950°; $[\phi]_{225}$ 0°; IR(KBr) 2·84 (—OH); 5·83 (6-ring C=O); 6·0, 11·8, 12·1 $\mu$ (—CH=C<); NMR: $\tau$9·17, 8·98, 8·95, 8·88, 8·73 (s, 15, 5 Me); 8·96 (d°, 3, $J$ = 5 Hz, Me); 8·37, 8·30 [bs, 6, >C=C(C$H_3$)$_2$]; 7·23† (m, 1, —COC$\underline{H}$C$H_2$—); 5·95 (m, 1, $w/2$ = 16 Hz, >C$\underline{H}$OH); 4·88, 4·70 (m, 2, 2 >C=C$\underline{H}$—). (Found: C, 81·87; H, 10·79; $M^+$; 440·365.‡ Calcd for $C_{30}H_{48}O_2$ (440·365): C, 81·76; H, 10·98%.)

*Kulinone acetate* (**1a**) To kulinone (50 mg) dissolved in pyridine (3 ml) was added $Ac_2O$ (0·2 ml), and the soln after being left to stand overnight at room temp was poured on ice. The cold mixture was neutralized with dil HCl, then extracted with ether and the ethereal layer washed with water and finally dried ($Na_2SO_4$). The residue was separated by preparative TLC (AB85) and gave the *acetate*; homogeneous by TLC but amorphous; $[\alpha]_D$ −5·5° (*c*, 1·7); IR($CS_2$) 5·7, 7·9 $\mu$ (—OAc); NMR (100 MHz); $\tau$9·16 (d°, 3, $J$ = 6 Hz, Me); 7·97 (s, 3, —OAc); 5·1 (m, 1, >C$\underline{H}$—OAc); 4·88, 4·70 (m, 2, 2 >C=C$\underline{H}$—); $M^+$, 482 ($C_{32}H_{50}O_3$ requires: 482); M-60, 422.

*Kulinone methanesulfonate* (**1b**). To a soln of kulinone (110 mg) in pyridine (3 ml) was added methanesulfonyl chloride (MsCl) (0·5 ml). After standing at room temp for 30 min the mixture was poured into ice-water, acidified with 5% HCl, and extracted with ether. The ether soln was washed with dil $NaHCO_3$ and water successively, and dried ($Na_2SO_4$). Removal of solvent afforded the *mesylate* (110 mg) as needles from MeOH; m.p. 152·0–152·5°; $[\alpha]_D$ −24° (*c*, 1·1); IR ($CS_2$) 7·43, 8·5, 10·4, 10·9, 11·55 $\mu$ (—OMs). (Found: C, 71·78; H, 9·85. Calcd for $C_{31}H_{50}O_4S$: C, 71·78; H, 9·72%.)

*Kulinone 3-ethylene ketal* (**1c**). To a soln of **1** (148 mg) in benzene (50 ml) was added ethylene glycol (1·5 ml) and *p*-toluenesulfonic acid (34 mg). After being heated at reflux for 8 hr, the mixture was cooled, and extracted with water. The benzene soln was washed with 5% $NaHCO_3$, then with water, and finally dried with calcium sulfate. Purification by preparative TLC (AB80) gave the *ketal* **1c** (135 mg) in amorphous form; IR($CS_2$) 2·8 (OH); 8·3, 8·96, 9·51 $\mu$ (ketal); NMR; $\tau$9·18 (9), 8·98, 8·78 (s, 15, 5 Me); 8·95 (d°, 3, $J$ = 4 Hz, Me); 8·38, 8·32 [bs, 6, >C=C(C$H_3$)$_2$]; 6·05 (s, 4, —OC$H_2$C$H_2$O—); 6·17–5·83 (m, 1, >C$\underline{H}$OH); 4·88, 4·72 (m, 2, 2 —CH=C<)

*Hydrogenation of kulinone*

1. In situ *with sodium borohydride*. Hydrogenation in a Brown[2] micro-analyzer (Delmar Scientific Laboratory)

*% yields are based on 5 g of residue B of the light petroleum extract.

†In every $\Delta^7$-3-keto derivative encountered in this work a distinctive 1-proton multiplet centered near $\tau$7·23 is present. In some instances the high field portion of the signal is obscured by other resonance, but at 100 MHz the whole signal may be shifted into view. One of these signals at 100 MHz was pictured in Ref. 1*b*, Fig 1.

Reference to this signal in the other $\Delta^7$-3-ketone will be omitted. Further details regarding this distinctive resonance will be included in an NMR paper on these compounds.

‡Molecular ions given to three decimal places are high resolution determinations.

with an uptake of 1 mole of hydrogen yielded 24,25-*dihydrokulinone*, prisms from light petroleum; m.p. 112–113°; $[\alpha]_D$ −23° (*c*, 0·9); IR($CS_2$) 2·73 (OH); 5·84 (6-membered ring C=O); 6·0, 11·92, 12·15 $\mu$ (>C=CH—); NMR: $\tau$9·17, 8·98, 8·97, 8·90, 8·73 (s, 15, 5 Me); 9·12 [d°, 6, $J$ = 6 Hz, $CH(C\underline{H}_3)_2$]; 8·97 (d°, 3, >$CHC\underline{H}_3$); 5·97 (m, 1, >C$\underline{H}$OH); 4·7 (m, 1, >C=C$\underline{H}$—). (Found: C, 81·22; H, 11·0; $M^+$, 442. Calcd for $C_{30}H_{50}O_2$ (442·38): C, 81·39; H, 11·38%.)

2. *Under neutral conditions.* Kulinone (733 mg) in MeOH (65 ml) was hydrogenated in a Parr apparatus with $PtO_2$ (200 mg) at atmospheric pressure for 30 min. The catalyst was filtered off and the filtrate evaporated to give a residue which by preparative TLC (AB80, 3X) gave 24,25-*dihydrokulinone* (**1d**, 630 mg).

A second product, *eupha-7-en-3β,16β-diol* (**5d**, 96 mg) crystallized as prisms from MeOH; m.p. 157·5–158·1°; $[\alpha]_D$ 0°; IR($CS_2$) 2·7, 2·8 $\mu$ (—OH); NMR: $\tau$9·23, 9·17, 9·13, 9·07, 9·03 (s, 15, 5 Me); 9·12 [d°, 6, $J$ = 6 Hz, —CH($C\underline{H}_3$)$_2$]; 8·96 (d°, 3, $J$ = 5 Hz, Me); 6·72 (m, 1, $w/2$ = 16 Hz, >C$\underline{H}$OH); 5·9 (m, 1, >C$\underline{H}$OH); 4·72 (m, 1, >C=C$\underline{H}$—); $M^+$, 444 ($C_{30}H_{52}O_2$ requires 444).

3. *Under acidic conditions.* Kulinone (150 mg) in glacial AcOH (20 ml) was shaken with $H_2$ and $PtO_2$ (65 mg) at atmospheric pressure for 35 hr. Filtration of catalyst and evaporation of solvent afforded *16β-hydroxyeuphenol* (**6d**, 145 mg) as rosettes of needles (from MeOH); m.p. 148·2–149·0°; $[\alpha]_D$ +16° (*c*, 1·0); IR($CHCl_3$) 2·71, 2·86 $\mu$ (OH); NMR: $\tau$9·23, 9·18, 9·03, 9·00, 8·87 (s, 15, 5 Me); 9·12 [d°, 6, $J$ = 6 Hz, —CH($C\underline{H}_3$)$_2$]; 6·73 (m, 1, >C$\underline{H}$OH); 5·87 (m, 1 > C$\underline{H}$OH); gives positive test with tetranitromethane. (Found: C, 81·17; H, 11·95; $M^+$, 444. Calcd for $C_{30}H_{52}O_2$ (444·40): C, 81·02; H, 11·79%)

Acetylation of **6d** followed by chromatography on Florisil and elution with light petroleum-ether (18:1) gave the *diacetate* (**6a**; amorphous; $[\alpha]_D$ +36° (*c*, 1·0); NMR: $\tau$9·22, 9·12 (6), 9·00, 8·95 (s, 15, 5 Me); 9·12 [d°, 6, —CH($C\underline{H}_3$)$_2$]; 7·98, 7·95 (s, 6, 2 —OAc); 5·45, 5·03 (m, 2, 2 >C$\underline{H}$—OAc).

*Butyrospermol* (**9**). To kulinone mesylate (**1b**, 165 mg) dissolved in anhyd ether (30 ml) was added a suspension of LAH (700 mg) in ether (50 ml). The mixture was heated under reflux for 34 hr. To the cooled mixture EtOAc was carefully added to destroy excess LAH; sat $Na_2SO_4$ aq was added until coagulation appeared and finally anhyd $Na_2SO_4$. Filtration of solid and evaporation of solvent afforded a residue (155 mg) which by preparative TLC (AB80) gave **9** (70 mg) as needles (from MeOH—$CH_2Cl_2$); m.p. 109·0–110·5°; $[\alpha]_D$ −11° (*c*, 4·0); IR(KBr) 2·87 (—OH), 6·0, 12·1 $\mu$ (>C=CH—); NMR: $\tau$9·23, 9·20, 9·15, 9·03 (6) (s, 15, 5 Me); 8·38, 8·33 [bs, 6, >C=C$\underline{M}$e$_2$]; 6·75 (m, 1, w/2 = 17 Hz, >C$\underline{H}$OH); 4·88, 4·73 (m, 2, 2 >C=C$\underline{H}$—); shown by mmp, TLC, IR, NMR, $M^+$ to be identical with an authentic sample of butyrospermol.* (lit.[13] m.p. 111–113°; $[\alpha]_D$ −12°). (Found: C, 83·97; H, 11·67; $M^+$, 426. Calcd for $C_{30}H_{50}O$ (426·70): C, 84·44; H, 11·81%.)

*Euphenol* (**10**). Butyrospermol (**9**, 24 mg) obtained from kulinone was hydrogenated in HOAc with $PtO_2$ catalyst at atmospheric pressure for 15 hr. Filtration of catalyst, evaporation of solvent, and purification by TLC afforded **10** (18·4 mg) as needles (from MeOH); m.p. 119·5–120·0°; $[\alpha]_D$ +17° (*c*, 2·8); IR(KBr) 2·89 $\mu$ (—OH); NMR: $\tau$9·23, 9·18, 9·11, 9·03, 8·98 (s, 15, 5 Me); 9·12 [d°, 6, $J$ = 6 Hz, >CH($C\underline{H}_3$)$_2$]; 6·75 (m, 1, $w/2$ = 17 Hz, >C$\underline{H}$OH). (lit[14] m.p. 122–123°; $[\alpha]_D$ +34°); identical with authentic euphenol† according to mmp, TLC, IR and NMR. (Found: C, 84·25; H, 12·28. Calcd for $C_{30}H_{52}O$: C, 84·04; H, 12·22%.)

*16-Dehydrokulinone* (**11**). A soln of kulinone (300 mg) in acetone (20 ml) was kept cool in an ice-bath, while Jones reagent was added until an orange color persisted. After 30 min the mixture was diluted with water and extracted with ether. The ether soln was washed with 5% $NaHCO_3$, then with water, and was dried ($Na_2SO_4$).

Evaporation of solvent afforded **11** (298 mg) as plates (from light petroleum); m.p. 108·0–190·0°; $[\phi]^D$ + 52° (*c*, 0·9); ORD (*c*, 0·1; MeOH): $[\phi]_{350}$ + 2,270°; $[\phi]_{316}$ + 5,670°; $[\phi]_{268}$ − 6,600°; $[\phi]_{231}$ −3,470°; $[\phi]_{225}$ − 5,080°; CD (*c*, 0·1; MeOH): $[\theta]$ −127°; $[\theta]_{307}$ +12,300°; $[\theta]_{251}$ +254°; $[\theta]_{225}$ +2,540°; $[\theta]_{215}$ 0°; IR($CS_2$) 5·74 (5-ring C=O), 5·82 $\mu$ (6-ring C=O); NMR: $\tau$9·00, 8·94 (6), 8·87, 8·80 (s, 15, 5 Me); 8·38, 8·32 [bs, 6, >C=C$\underline{M}$e$_2$]; 8·93 (d°, 3, $J$ = 4 Hz, Me); 4·88, 4·63 (m, 2, 2 >C=C$\underline{H}$—). (Found: C, 82·64; H, 10·47; $M^+$, 438. Calcd for $C_{30}H_{46}O_2$ (438·67): C, 82·14; H, 10·57%.)

*16-Dehydrokulinone 3-ethylene ketal* (**12c**) was prepared by ketalization of **12** (245 mg). TLC indicated a small amount of starting material remained with the 3-ketal. Evaporation of solvent and purification by preparative TLC (AB80 2X) afforded the product (200 mg). Further purification by column chromatography on Florisil (15 g) eluted with light petroleum-ether (18:1) yielded homogeneous **12c** (150 mg); amorphous; ORD (*c*, 0·24; MeOH): $[\phi]_{600}$ +345°; $[\phi]_{589}$ +365°; $[\phi]_{332}$ +11,300°; $[\phi]_{324}$ +7,700°; $[\phi]_{320}$ +8,720°; $[\phi]_{312-310}$ +324° (shoulder); $[\phi]_{296}$ −7,300° (inflection); $[\phi]_{283}$ −9,600°; $[\phi]_{229}$ 0°; IR($CS_2$) 5·74 (5-ring C=O); 8·95, 8·57, 8·73, 10·22, 10·51 $\mu$ (ketal); NMR (100 MHz): $\tau$9·18 (6), 9·01, 8·98, 8·84 (s, 15, 5 Me); 8·95 (d°, 3, $J$ = 7 Hz, Me); 8·39, 8·31 [bs, 6, >C($CH_3$)$_2$]; 4·91, 4·74 (m, 2, 2 >C=C$\underline{H}$—); 6·04 (s, 4, —$OCH_2CH_2O$—); $M^+$, 482 ($C_{32}H_{50}O$ requires 482); M-99‡ 383.

*16-Dehydrokulinone 3-ethylene ketal* (**12c**) was also prepared by Jones oxidation of kulinone 3-ethylene ketal (**1c**). The product was identical with that prepared above according to IR and NMR.

*16-Hydroxybutyrospermol* (**5**). A soln of kulinone (100 mg) and $NaBH_4$ (100 mg) in MeOH (50 ml) was magnetically stirred at room temp for 30 min. Water was added to the mixture which was then extracted with ether. The ethereal soln was washed thoroughly with water and was dried ($Na_2SO_4$). Evaporation yielded **5** (99 mg) as prismatic needles (from MeOH); m.p. 132·0–132·5°; $[\alpha]_D$ +4°; (*c*, 1·1); IR ($CHCl_3$) 2·73, 2·88, 9·7, 10·15 $\mu$ (OH); NMR: $\tau$9·25, 9·17, 9·15, 9·03, 8·78 (s, 15, 5 Me); 8·97 (s, 3, >CH—C$\underline{H}_3$); 8·96 (d°, 3, $J$ = 5 Hz, Me); 8·38, 8·32 [bs, 6, >C=C($CH_3$)$_2$]; 6·75 (m, 1, $w/2$ = 17 Hz, >C$\underline{H}$OH); 5·93 (m, 1, >C$\underline{H}$OH); 4·88, 4·73 (m, 2, 2 >C=C$\underline{H}$—); $M^+$, 442 ($C_{30}H_{50}O_2$ requires: 442).

Acetylation of **5** followed by chromatography on Florisil and elution with light petroleum-ether (20:1) yielded *eupha-7,24-dien-3β,16β-diol diacetate* (**5a**); amorphous; $[\alpha]_D$ +33° (*c*, 1·3); IR($CS_2$) 5·73, 8·02 $\mu$

*We are indebted to Professor K. G. Lewis (Univ. of New England) for a reference sample of butyrospermol.

†We are indebted to Professor J. Fried (Univ. of Chicago) for a reference sample of euphenol.

‡A M-99 fragment is often found in steroidal 3-ketals (H. Budzikiewicz, C. Djerassi and D. H. Williams, *Structure Elucidation of Natural Products by Mass Spectrometry* p. 40. Holden-Day (1964).

(—OAc); NMR: τ7·98, 7·95 (s, 6, 2 —OAc); 5·43 (m, 1, >CH—OAc); 5·07 (m, 1, >CH—OAc).

Catalytic hydrogenation of 16β-hydroxybutyrospermol (**5**) in MeOH afforded the *dihydro derivative*, identical to *eupha-7-en-3β,16β-diol* (**5d**) according to IR and NMR.

*16-Epikulinone* (**12**). 16-Dehydrokulinone 3-ketal (**11a**, 200 mg) was reduced with LAH (220 mg) in ether for 2 hr. Workup gave a ketal mixture which could not be separated by crystallization nor by preparative TLC, but after hydrolysis of the ketals, the ketones were separable. By preparative TLC, followed by crystallization, the ketones were separated and shown to be **12** (80 mg) and kulinone (64 mg). *16-Epikulinone* crystallized as thin plates from MeOH; m.p. 173–174·5°; $[\alpha]_D$ −47° (*c*, 3·1); NMR: τ8·97 (9), 8·93, 8·87 (s, 15, 5 Me); 8·98 (d°, 3, $J$ = 5 Hz, Me); 8·35, 8·28 [bs, 6, >C=C(CH$_3$)$_2$]; 5·53 (m, 1, $w/2$ = 21 Hz, >CHOH); 4·83, 4·63 (m, 2, 2 >C=CH—). (Found: C, 81·90; H, 10·89; M$^+$, 440·366. Calcd for $C_{30}H_{48}O_2$ (440·365): C, 81·76; H, 10·98%.)

*16-Epikulinone acetate* (**12a**) was prepared by acetylation of **12** and purified by preparative TLC (AB80); amorphous; $[\alpha]_D$ −90° (*c*, 3·1); NMR: τ8·98 (6), 8·95 (6), 8·88 (s, 15, 5 Me); 9·16 (d°, 3, $J$ = 5 Hz, Me); 8·37, 8·28 [bs, 6, >C=CMe$_2$]; 7·98 (s, 3, —OAc); 4·97–4·63 (m, 3, >CHOAc, 2 >C=CH—).

*16-Epikulinone 3-ketal* (**12c**) was prepared by ketalization of **12**; amorphous, NMR: τ9·22, 9·17, 9·03, 8·98 (6), (s, 15, 5 Me); 8·98 (d°, 3, $J$ = 5 Hz, Me); 8·37, 8·30 [bs, 6, >C=CMe$_2$]; 6·03 (s, 4, —OCH$_2$CH$_2$O—); 5·53 (m, 1, $w/2$ = 20 Hz, >CHOH); 4·87, 4·70 (m, 2, 2 >C=CH—).

*Kulactone* (**2**) recrystallized from MeOH as prismatic rods; m.p. 163·0–164·5°; $[\alpha]_D$ −60° (*c*, 1·0); ORD (*c*, 0·14; MeOH): $[\phi]_{600}$ −250°; $[\phi]_{589}$ −264°; $[\phi]_{313}$ −2,660°; $[\phi]_{278}$ −240°; $[\phi]_{231}$ −7,850°; $[\phi]_{225}$ −6,600°; CD (*c*, 0·1; MeOH): $[\theta]_{400}$ −89°; $[\theta]_{385}$ −445°; $[\theta]_{370-330}$ 0°; $[\theta]_{293}$ −2,800°; $[\theta]_{255}$ −445°; $[\theta]_{211}$ −25,000°; $[\theta]_{207}$ −21,000°: IR(KBr) 6·0, 11·9, 12·1 (>C=CH—); 5·83 (6-ring C=O); 5·59, 10·49 μ (γ-lactone); NMR: τ9·03, 8·97, 8·94, 8·88, 8·73 (s, 15, 5 Me); 8·36, 8·30 [bs, 6, >C=CMe$_2$]; 7·23 (m, 1); 5·83 (m, 1, >CHO—); 4·88, 4·63 (m, 2, 2 >C=CH—). (Found: C, 79·54; H, 9·56; M$^+$, 452·327. Calcd for $C_{30}H_{44}O$ (452·329): C, 79·60; H, 9·80%.)

*Hydrogenation of kulactone*

1. In situ *with sodium borohydride*. In a Brown[2] microanalyzer kulactone took up one mole of $H_2$ and formed 24,25-dihydrokulactone **2d**, which crystallized from MeOH as prisms; m.p. 144·5–145·5°; $[\alpha]_D$ −63° (*c*, 1·2); IR(CS$_2$) 5·58, 8·68, 10·48 (γ-lactone); 5·84 (6-ring C=O); 6·0, 11·9, 12·12 μ (>C=CH—); NMR: τ9·12 [d°, $J$ = 6 Hz, >CH(CH$_3$)$_2$]. (Found: C, 79·14; H, 10·23. Calcd for $C_{30}H_{46}O_3$: C, 79·25; H, 10·20%.)

2. *Under neutral conditions*. Kulactone (422 mg) in MeOH (30 ml) with $PtO_2$ (70 mg) was hydrogenated in a Parr apparatus at atmospheric pressure for 30 min. Filtration of catalyst and evaporation of solvent gave a solid residue which consisted of two products according to TLC (AB80). Separation was effected by preparative TLC (AB80, 2X). The product with higher $R_f$ was 24,25-*dihydrokulactone* (**2d**, 273 mg), identical with the product of part 1.

The other product, 24,25-*dihydro-3-epikulolactone* (**14d**, 110 mg), did not crystallize; $[\alpha]_D$ −38° (*c*, 1·0); IR (CS$_2$) 2·71 (OH); 5·59, 8·68, 10·46 μ (γ-lactone); NMR: τ9·23, 9·03 (6), 9·13, 8·78 (s, 15, 5 Me); 9·12 [d°, 6, $J$ = 6 Hz, —CH(CH$_3$)$_2$] 6·72 (m, 1, >CHOH); 5·82 (m, 1, >CHO—); 4·67 (m, 1, >C=CH—).

*Reduction of kulactone with* $NaBH_4$

To a soln of kulactone (200 mg) in MeOH (50 ml) was added $NaBH_4$ (30 mg). After being magnetically stirred for 2 hr at room temp, the mixture was processed, as described in the reduction of kulinone. Preparative TLC of the residue (AB80, 6X) furnished two principal products. The first (132 mg) was 3-epikulolactone (**14**); amorphous; $[\alpha]_D$ −38° (*c*, 1·9); IR(CS$_2$) 2·72 (OH); 5·59, 10·49 μ (γ-lactone); NMR: τ9·22, 9·15, 9·07, 9·05, 8·80 (s, 15, 5 Me); 8·37, 8·28 [bs, 6, >C=C(CH$_3$)$_2$]; 6·73 (m, 1, $w/2$ = 16 Hz, >CHOH); 5·80 (m, 1, >CHOH); 4·88, 4·7 (m, 2, 2 >C=CH—).

Acetylation of **14** gave *3-epikulolactone acetate* (**14a**) as thin plates (from MeOH); m.p. 161–163·0°; $[\alpha]_D$ −13° (*c*, 1·8); IR(CS$_2$) 5·58, 10·48 (γ-lactone); 5·74, 8·05 μ (acetate); NMR: τ9·22, 9·15, 9·07, 9·05, 8·80 (s, 15, 5 Me); 8·38, 8·30 [bs, >C=CMe$_2$]; 8·00 (s, 3, —OAc—); 5·83 (m, 1, $w/2$ = 30 Hz, >CHO—); 5·88 (m, 1, $w/2$ = 18 Hz, >CHOAc); 4·87, 4·72 (m, 2, 2 >C=CH—). (Found: C, 77·30; H, 9·96. Calcd for $C_{32}H_{48}O_4$: C, 77·38; H, 9·44%.)

The second product (51 mg) was *3β,16β-dihydroxy-eupha-7,24-dien-21-oic acid methyl ester* (**25**), as rosettes (from MeOH); m.p. 92–94°; $[\alpha]_D$ −5° (*c*, 1·6); IR(CHCl$_3$) 2·72, 2·80 (OH); 5·81 μ (ester); NMR: τ9·23, 9·15, 9·13, 9·03, 8·75 (s, 15, 5 Me); 8·40, 8·30 [bs, 6, >C=C(CH$_3$)$_2$]; 6·75 (m, 1, $w/2$ = 18 Hz, >CHOH); 6·28 (s, 3, —COOCH$_3$); 6·00 (m, 1, $w/2$ = 17 Hz, >CHOH); 4·90, 4·7 (m, 2, 2 >C=CH—).

Acetylation of **25** (40 mg) with $Ac_2O$ pyridine at room temp for 24 hr, workup, and preparative TLC gave the diacetate (*3β,16β-diacetoxyeupha-7,24-dien-21-oic acid methyl ester*) (**25a**, 34 mg); amorphous; IR(CS$_2$) 5·73 (acetate and ester); 8·04 μ (acetate); NMR: τ9·22, 9·13 (6), 9·07, 8·86 (s, 15, 5 Me); 8·42, 8·30 [bs, 6, >C=CMe$_2$]; 8·00, 7·95 (s, 6, 2, —OAc) 6·40 (s, 3, —COOCH$_3$); 5·45 (m, 1, >CHOAc); 5·12–4·72 (m, 3, >CHOAc, 2 >C=CH—).

Kulactone 3-ethylene ketal (**2c**). Ketalization of kulactone (320 mg) afforded the ketal **2c** (310 mg) as long needles (from MeOH); m.p. 194·0–195·5°; $[\alpha]_D$ −40° (*c*, 2·0); ORD (*c*, 0·07; MeOH): $[\phi]_{600}$ −150°; $[\phi]_{589}$ −210°; $[\phi]_{232}$ −7,000°; $[\phi]_{220}$ −1,200°; IR(CHCl$_3$) 5·59, 8·64 10·48 (γ-lactone); 8·3, 8·92, 9·01, 9·25, 9·6, 9·81 μ (ketal); NMR: τ9·20, 9·18, 9·05, 8·98, 8·78, (s, 15, 5 Me); 8·38, 8·30 [bs, 6, >C=CMe$_2$]; 6·05 (s, 4, —OCH$_2$CH$_2$O—); 4·88, 4·70 (m, 2, 2 >C=CH—). (Found: C, 77·20; H, 9·78. Calcd for $C_{32}H_{48}O_4$: C, 77·38; H, 9·74%.)

*Kulonic acid* (**23**)

Kulactone (165 mg) and KOH (500 mg) in 25 ml MeOH water (4:1) were heated under reflux for 8 hr. The cooled mixture was acidified with 5% HCl and extracted thoroughly with ether. The ethereal soln was washed with water to neutrality and dried ($Na_2SO_4$). Evaporation of the solvent gave **23** contaminated with traces of *methyl ester* (**4**, see below), as shown by TLC (AB80 and ABJ 50:48) Crystallization afforded pure *acid* **23** (152 mg) as prismatic needles (from MeOH); m.p. 205–207·0°; $[\alpha]_D$ −38° (*c*, 1·3); ORD (*c*, 0·06; MeOH): $[\phi]_{350}$ −570°; $[\phi]_{311}$ −1,580°; $[\phi]_{282}$ +1,600°; $[\phi]_{250-230}$ +1,380°; $[\phi]_{225}$ 0°; IR(CHCl$_3$) 2·88, 3·0–4·2 (broad), 5·86, 10·7 μ (—COOH); NMR: τ9·15, 8·97, 8·93, 8·87, 8·73 (s, 15, 5 Me); 8·38, 8·30 [bs, 6, >C=CMe$_2$]; 5·87 (m, 1, >CHOH); 4·87, 4·67 (m, 2, 2 >C=CH—); 3·12 (b, 1, —COOH; disappears on addition of $D_2O$). (Found: C, 76·61, H, 10·00; M$^+$, 470. Calcd for $C_{30}H_{46}O_4$ (470·67): C, 76·55; H 9·85%.)

Esterification of **23** with excess diazomethane afforded methyl ester which by ketalization gave a ketal as thin prismatic plates (from MeOH); m.p. 173–174·5°; $[\alpha]_D$ −14° (*c*, 0·9); ORD (*c*, 0·13; MeOH): $[\phi]_{350}$ +20°; $[\phi]_{234}$ +1,420°; $[\phi]_{221}$ −393°; shown to be identical with *methyl kulonate* 3-*ethylene ketal* (**4c**, see below).

*Attempted lactonization of kulonic acid*

1. *With dicyclohexylcarbodiimide.* (a) By procedure of Woodward *et al.*[27] To a pyridine solution (2 ml) of **23** (5 mg) was added dicyclohexylcarbodiimide (15 mg). The mixture was heated under reflux for 2 hr in an atmosphere of $N_2$. After being cooled, the mixture was examined by TLC and no spot corresponding to that of kulactone was observed.(b) By modified method of Johnson *et al.*[28] A soln of **23** (2 mg) and dicyclohexylcarbodiimide (15 mg) in pyridine (2 ml) stood at room temp. The reaction was monitored by TLC; (up to 10 days) no kulactone was detected in the mixture.

2. *With p-toluenesulfonic acid.* A mixture of **23** (6 mg), *p*-TsOH (8 mg), and xylene (3 ml) was boiled under reflux for 30 min in an atmosphere of $N_2$, as described by Johnson *et al.*[28] The cooled mixture did not show the presence of kulactone by TLC.

*Methyl kulonate* (**4**)

1. *With methanolic* HCl. Kulactone (200 mg) in MeOH (15 ml) and conc HCl (2 drops) was warmed on a hot plate for 20 min. The cooled mixture was diluted with water and extracted with ether. The ethereal soln was washed with 5% $NaHCO_3$ and water successively, dried ($Na_2SO_4$), and evaporated. Preparative TLC of the residue (AB80, 4X) afforded **4** (175 mg) as very fine needles (from light petroleum); m.p. 107·8–108·5°; $[\alpha]_D$ −32° (*c*, 0·9); IR($CS_2$) 2·78 (—OH); 5·79, 8·31, 8·5 $\mu$ (—$COOCH_3$); NMR: $\tau$9·17, 9·00, 8·97, 8·90, 8·73 (s, 15, 5 Me); 8·42, 8·32 [bs, 6, >C=$CMe_2$]; 6·30 (s, 3, —$COOCH_3$); 6·02 (m, 1, >C<u>H</u>OH); 4·92, 4·67 (m, 2, 2 >C=C<u>H</u>—). (Found: C, 76·73; H, 9·96; $M^+$ 384, (no significant peak at M—$CO_2$Me). Calcd for $C_{31}H_{48}O_4$ (384·69): C, 76·82, H, 9·98%.)

2. *With methanolic* KOH. Kulactone (150 mg) in 0·1% methanolic KOH (25 ml) was warmed gently. Removal of the solvent and trituration with ether gave the product **4** (150 mg), according to TLC, GLC, IR and NMR.

3. *From recrystallization of kulactone.* During continued recrystallization of kulactone from MeOH, a new compound was gradually concentrated in the mother liquors. By TLC and GLC the material was shown to be **4**.

4. *From total plant extract.* Methyl kulonate was originally found in the mother liquors from methanolic recrystallizations of fractions rich in kulactone. To confirm the presence of the ester in original plant material, the entire chromatographic separation and subsequent isolation of methyl kulonate were carried out in the absence of MeOH or any methylating solvents.

*Methyl kulonate acetate* (**4a**). Acetylation of methyl kulonate followed by chromatography on Florisil and elution with light petroleum-ether (10:1) afforded the **4a**; amorphous; IR($CS_2$) 5·7, 8·0 $\mu$ (acetate); NMR: $\tau$9·13, 8·98, 8·95, 8·88, 8·81 (s, 15, 5 Me); 8·42, 8·31 [bs, 6, >C=$CMe_2$]; 8·00 (s, 3, —OAc); 6·4 (s, 3, —$COOCH_3$); 4·98 (m, 1, >C<u>H</u>OAc); 4·92, 4·70 (m, 2, 2 >C=C<u>H</u>—).

*Methyl kulonate mesylate* (**4b**). Mesylation of methyl kulonate and workup gave the *mesylate*; amorphous; IR($CS_2$) 7·4, 8·44, 10·75 $\mu$ (—OMs).

*Methyl kulonate 3-ethylene ketal* (**4c**) was prepared by ketalization of the ester **4**. Workup and purification by preparative TLC (AB80, 3X) afforded the *product* as thin prismatic plates (from MeOH); m.p. 173·5–174·5°; $[\alpha]_D$ −13·5°; (*c*, 0·9); ORD (*c*, 0·16; MeOH): $[\phi]_{600}$ −33°; $[\phi]_{589}$ −46°; $[\phi]_{235}$ +1,330°; $[\phi]_{225}$ 0°; IR($CS_2$) 8·32, 8·98, 9·34, 9·58, 9·8 $\mu$ (—$OCH_2CH_2O$—); NMR: $\tau$9·18, 9·17 (6), 8·98, 8·77 (s, 15, 5 Me); 8·40, 8·30 [bs, 6, >C=C($CH_3$)$_2$]; 6·23 (s, 3, —COOMe); 6·03 (s, 4, —$OCH_2CH_2$—; m, 1, >C<u>H</u>OH); 4·88, 4·70 (m, 2, 2 >C=C<u>H</u>—). (Found: C, 75·18; H, 9·61. Calcd for $C_{33}H_{52}O_5$: C, 74·96; H, 9·91%.)

*Methyl kulonate* 2,4-*dinitrophenylhydrazone* (**4e**). When kulactone (90 mg) in MeOH (15 ml) was treated with 2,4-dinitrophenylhydrazine (42 mg) and conc HCl (5 drops), the product turned out to be the 2,4-*dinitrophenylhydrazone* of *methyl kulonate* (**4e**), which crystallized from MeOH as long yellow needles; m.p. 214–215·0°; IR($CHCl_3$); 2·98 (NH); 6·60, 7·5 (—$NO_2$); 2·8 (—OH); 5·83 $\mu$ (ester); NMR: $\tau$9·17, 9·0, 8·78, 8·72 (6) (s, 15, 5 Me); 8·4, 8·32 [bs, 6, >C=C(C<u>H</u>$_3$)$_2$]; 6·28 (s, 3, —COOMe); 5·98 (m, 1, >C<u>H</u>OH); 4·90, 4·65 (m, 2, 2 >C=C<u>H</u>—); 1·98 (s, 1); 1·7 (d, 1, $J$ = 2·5 Hz); 0·82 (d, 1, $J$ = 3 Hz); −1·33 (bs, 1); compatible with the assignment of a 2,4-dinitrophenylhydrazone.[42]

*Methyl* 16-*dehydrokulonate* (**15**). Methyl kulonate (**4**, 178 mg) in acetone (20 ml) was oxidized with Jones reagent to yield 166 mg of crude product. Chromatography on Florisil and elution with light petroleum-ether (8:1) afforded **15**; amorphous, but homogeneous on TLC; $[\alpha]_D$ +37° (*c*, 1·4); ORD (*c*, 0·11; MeOH): $[\phi]_{350}$ +1,450°; $[\phi]_{318}$ +4,520°; $[\phi]_{310}$ +3,980° (inflection); $[\phi]_{280}$ −4,560°; $[\phi]_{234}$ +1,540° (inflection); $[\phi]_{227}$ +1,980°; $[\phi]_{223}$ +990°; CD (*c*, 0·11; MeOH): $[\theta]_{350}$ +36°; $[\theta]_{304}$ +7,300° (inflection); $[\theta]_{298}$ +7,400°; $[\theta]_{247}$ +307°; $[\theta]_{230}$ +2,080°; IR($CS_2$) 5·70 (5-ring C=O and ester); 5·82 $\mu$ (6-ring C=O); NMR: $\tau$9·04, 8·94 (6), 8·87, 8·74 (s, 15, 5 Me); 8·40, 8·31 [bs, 6, > C=C$Me_2$]; 7·77 (s, 2, ⩾C<u>H</u>$_2$CO); 6·23 (s, 3, —COOMe); 4·9, 4·62 (m, 2, 2 >C=C<u>H</u>—).

*Methyl* 16-*dehydrokulonate* 3-*ethylene ketal* (**15c**)

1. *Methyl* 16-*dehydrokulonate* (**15**, 113 mg) was ketalized with ethylene glycol and *p*-TsOH. Workup and purification by preparative TLC (AB80) gave **15c**; amorphous; $[\alpha]_D$ +48° (*c*, 1·3); ORD (*c*, 0·13; MeOH); $[\phi]_{350}$ +1,950°; $[\phi]_{322}$ +4,540° (inflection); $[\phi]_{312}$ +5,280°; $[\phi]_{268}$ −4,860°; $[\phi]_{229}$ +2,000°; $[\phi]_{220}$ −730°; IR($CS_2$) 8·35, 8·99, 9·26 (—$OCH_2CH_2O$—); 5·72 $\mu$ (5-ring C=O and ester superimposed); NMR: $\tau$9·17 (6), 9·05, 8·97, 8·78 (s, 15, 5 Me); 8·39, 8·30 [bs, 6, >C=$CMe_2$]; 7·79 (s, 2, ⩾C<u>H</u>$_2$C=O); 7·17 (d°, 1, $J$ = 11 Hz, 17-H); 6·23 (s, 3, —COOMe); 6·02 (s, 4, —$OCH_2CH_2O$—); 4·88, 4·68 (m, 2, 2 >C=C<u>H</u>—); $M^+$, 526 ($C_{33}H_{50}O_5$ requires 526).

2. Methyl kulonate 3-ethylene ketal (**4c**, 190 mg) on oxidation with Jones reagent yielded a product (180 mg), identical with **15c**, above, according to IR and NMR.

16$\beta$,21-*Dihydroxyeupha*-7,24-*dien*-3-*one*-3-*ethylene ketal* (**16c**)

1. *From kulactone* 3-*ethylene ketal* (**2c**). To an ethereal soln (50 mg) of **2c**, (212 mg) was added a suspension of LAH (330 mg) in ether (50 ml). The mixture was boiled under reflux for 2 hr. Workup afforded the product **16c** (200 mg) as long needles (from MeOH); m.p. 172·0–172·5°; $[\alpha]_D$ −11° (*c*, 2·1); ORD (*c*, 0·16; MeOH); $[\phi]_{600}$ −60°; $[\phi]_{589}$ −67°; $[\phi]_{233}$ +1,470°; $[\phi]_{225}$ +740°; IR($CHCl_3$) 2·7, 2·85, 9·85, 10·04 $\mu$ (OH); NMR: $\tau$9·2, 9·18 (6), 8·98,

8·77 (s, 15, 5 Me); 8·38, 8·32 [bs, 6, >C=CMe$_2$]; 6·18 (b, 2, —CH$_2$OH); 5·80 (m, 1, —CHOH); 4·87, 4·75 (m, 2, 2 >C=CH—).(Found: C, 77·06; H, 10·25. Calcd for $C_{32}H_{52}O_4$: C, 76·75; H, 10·47%.)

2. *From methyl kulonate* 3-*ketal* (**4c**). Reduction of **4c** (150 mg) with LAH (400 mg) as in part 1, afforded the identical *product* **16c** (122 mg), according to mmp, IR, TLC, and NMR.

3. *From methyl* 16-*dehydrokulonate* 3-*ketal* **15c** (240 mg) by reduction with LAH in ether for 2 hr, two products were formed. After workup the residue was separated by preparative TLC and recrystallized to give 20 mg of **16c**, and 35 mg of its 16$\alpha$-epimer (see below).

16$\beta$,21-*Dihydroxyeupha*-7,24-*dien*-3-*one ketal diacetate* (**16f**)

Acetylation afforded the ketal *diacetate* (**16f**) as fine needles (MeOH); m.p. 112·0–113·5°; $[\alpha]_D$ +21° (*c*, 1·5); IR($CS_2$) 5·71, 8·00 $\mu$ (—OAc); NMR: $\tau$9·20, 9·18, 9·13, 8·98, 8·85 (s, 15, 5 Me); 8·40, 8·30 [bs, 6, >C=CMe$_2$]; 8·02, 7·97 (s, 6, 2, —OAc); 6·03 (s, 4, —OCH$_2$CH$_2$O—); 5·96 (d, 2, $J$ = 3 Hz, >CHCH$_2$OAc); 5·03 (m, 1, >CH—OAc); 4·9, 4·75 (m, 2, 2 >C=CH—). (Found: C, 73·69; H, 9·68. Calcd for $C_{36}H_{56}O_6$: C, 73·93; H, 9·65%.)

16$\beta$,21-*Dihydroxyeupha*-7,24-*dien*-3-*one* (**16**) was prepared by warming on a hot plate for 30 min the 3-ketal (**16c**) in 90% HOAc. Removal of solvent and purification by preparative TLC (AB80, 5X) afforded the *product* **16** as prismatic rods (from $CH_3OH$); m.p. 160·0–161·0°: $[\alpha]_D$ −30° (*c*, 0·8); IR($CHCl_3$) 2·71, 2·86, 8·99, 9·85, 10·04 (OH); 5·84 $\mu$ (6-ring C=O); NMR: $\tau$9·17, 8·98, 8·95, 8·88, 8·72 (s, 15, 5 Me); 8·37, 8·30 [bs, >C=CMe$_2$]; 6·15 (bs, 2, >CHCH$_2$OH); 5·77 (m, 1, >CHOH); 4·87, 4·67 (m, 2, 2 >C=CH—). (Found: C, 78·94; H, 10·56. Calcd for $C_{30}H_{48}O_3$: C, 78·90; H, 10·59%.)

Acetylation of **16** followed by preparative TLC (AB80) afforded 16$\beta$,21-*dihydroxyeupha*-7,24-*dien*-3-*one diacetate* (**16a**) as prisms (from MeOH); m.p. 103·5–104·4°; $[\alpha]_D$ 0° (*c*, 1·0); NMR: $\tau$9·13, 8·98, 8·94, 8·88, 8·80 (s, 15, 5 Me); 8·40, 8·30 [bs, 6, >C=CMe$_2$]; 8·01, 7·96 (s, 6, 2 —OAc); 6·12 (d, 2, $J$ = 3 Hz > CHCH$_2$OAc); 4·95, 4·70 (m, 2, 2 >C=CH—).

*Conversion of kulactone to kulinone*

To a pyridine soln (10 ml) of **16c** (250 mg), prepared from **2c**, was added a soln of MsCl (135 mg) in pyridine (3 ml) in two portions. After 2 hr at room temp the mixture was processed, as described for **1b**, to give 237 mg of residue which still contained about 65% of unchanged diol ketal **16c**, according to TLC estimation.

The partially mesylated mixture (180 mg) without separation was reduced with LAH (530 mg). Workup and preparative TLC (AB80) of the residue (155 mg) furnished 39 mg of *kulinone ketal* (**1c**), indicated by the NMR spectrum. On treatment with 90% HOAc, the ketal was converted into *kulinone* (**1**), identical with the naturally occurring sample according to optical rotations, m.p., m.m.p., IR, and NMR. The starting diol ketal **16c** (89 mg) was recovered (examined by $[\alpha]_D$, IR and NMR).

16-*Epikulactone* (**17**)

1. A soln of **4b** (110 mg) in collidine (25 ml) was heated under reflux for 3 hr. The cooled mixture was poured into ice-water, acidified with 5% HCl, and extracted with ether. The ethereal soln was washed with water, 5% $NaHCO_3$, and water successively, dried ($Na_2SO_4$), and evaporated to a dark brown residue. Purification by preparative TLC (AB80, 3X) yielded **17** (47 mg) as needles (from MeOH); m.p. 182·0–183·0°; $[\alpha]_D$ 0° (*c*, 1·0); ORD (*c*, 0·1; MeOH): $[\phi]_{370-362}$ 0°; $[\phi]_{345}$ +95°; $[\phi]_{314}$ −760°; $[\phi]_{248}$ +4,280° (inflection); $[\phi]_{220}$ +6,850°; CD (*c*, 0·10; MeOH): $[\theta]_{370-332}$ 0°; $[\theta]_{290}$ −2,570°; $[\theta]_{224}$ +475°; $[\theta]_{207}$ −5,700°; $[\theta]_{203}$ 0°; IR($CS_2$) 5·61, 8·51 ($\gamma$-lactone); 5·82 $\mu$ (6-ring C=O), NMR: $\tau$9·08, 8·98, 8·95 (6), 8·88 (s, 15, 5 Me); 8·37, 8·32 [bs, 6, >C=CMe$_2$]; 4·97 (m, 1, >CHOH); 4·88, 4·62 (m, 2, 2 >C=CH—). (Found: C, 79·74; H, 9·98; M$^+$, 452·330. Calcd for $C_{30}H_{44}O_3$ (452·329): C, 79·60; H, 9·80%.)

2. To a soln of **4** (390 mg) in pyridine (20 ml) was added MsCl (0·5 ml). After standing for 30 min at room temp the mixture was heated under reflux for 1 hr. Workup as in part 1 gave a dark brown residue which was chromatographed on Florisil. Elution with light petroleum-ether (1:1) yielded **17** (270 mg), identical with the product obtained above, according to mmp, TLC, GLC, IR and NMR comparisons.

24,25-*Dihydro*-16-*epikulactone* (**17d**)

1. 16-Epikulactone was hydrogenated in MeOH soln and $PtO_2$ as catalyst to yield **17d**, crystallized from MeOH as needles; m.p. 195·5–196·5°, $[\alpha]_D$ −4° (*c*, 1·3); IR($CS_2$) 5·61, 8·51 ($\gamma$-lactone); 5·82 $\mu$ (6-ring C=O); NMR: $\tau$9·12 [d°, 6, $J$ = 6 Hz, —CHMe$_2$]. (Found: C, 79·62; H, 10·12. Calcd for $C_{30}H_{46}O_3$: C, 79·25; H, 10·20%.)

2. Methyl kulonate **4** was hydrogenated as in part 1 above, and without isolation (after examination by NMR) the crude dihydro product was mesylated. The mesylate was heated in collidine as in the preparation of **17** above and processed similarly. The crystallized product was found to be identical with **17d**, according to IR and NMR analyses.

16-*Epikulactone* 3-*ethylene ketal* (**17c**)

1. 16-Epikulactone (**17**) was ketalized to give **17c** as prismatic plates (from MeOH); m.p. 207·0–208·2°; $[\alpha]_D$ +22° (*c*, 1·2); IR($CHCl_3$) 5·65, 8·47 ($\gamma$-lactone); 8·98, 9·6 $\mu$ (ketal); NMR: $\tau$9·20, 9·17, 9·08, 9·00, 8·98 (s, 15, 5 Me); 8·35, 8·28 [bs, 6, >C=C(CH$_3$)$_2$]; 6·03 (s, 4, —OCH$_2$CH$_2$O—); 4·98 (m, 1, —CHOOC—); 4·88, 4·67 (m, 2, 2 >C=CH—). (Found: C, 77·78; H, 9·76. Calcd for $C_{32}H_{48}O_4$: C, 77·38: H, 9·74%.)

2. *Methyl kulonate* 3-*ketal* (**4c**, 387 mg) was mesylated, and the crude mesylate, on being heated in collidine and processed, yielded **4c**, identical with the product of part 1, according to IR and NMR.

3. To a soln of **15c** (100 mg) in MeOH (50 ml) was added $NaBH_4$ (200 mg). The mixture was magnetically stirred for 4 hr at room temp and processed. Preparative TLC (AB80) of the residue gave two fractions:

The first fraction (62 mg), on crystallization, gave a crystalline compound (30 mg), identical with **17c** according to IR and NMR spectra. On cleavage, **17c** yielded a *keto derivative* which was identical with **17** by comparison of m.p., IR, NMR, and $[\alpha]_D$.

The second fraction (35 mg), homogeneous on TLC, was separated as thin prismatic plates from MeOH. The product was shown to be identical with *methyl kulonate* 3-*ethylene ketal* (**4c**) by direct comparison.

16$\alpha$,21-*Dihydroxyeupha*-7,24-*dien*-3-*one ethylene ketal* (**18c**)

1. To a soln of **17c** (280 mg) in ether (50 ml) was added a suspension of LAH (500 mg) in ether (50 ml). The mix-

ture was then heated under reflux for 2 hr. Workup and preparative TLC (AB80, 4X) yielded **18c**; amorphous: $[\alpha]_D$ −11° (*c*, 1·0); IR($CHCl_3$) 3·00 (broad, OH); 8·31, 8·98, 9·66 $\mu$ (ketal); NMR: $\tau$9·22, 9·17, 9·02, 8·98, 8·95 (s, 15, 5 Me); 8·37, 8·30 [bs, 6, >C=C($CH_3$)$_2$]; 6·22 (m, 2, >CHC$\underline{H}_2$OH); 6·03 (s, 4, —$OCH_2CH_2O$—); 5·50 (m, 1, >C$\underline{H}$OH); 4·90, 4·72 (m, 2, >C=C$\underline{H}$—).

2. Methyl 16-dehydrokulonate 3-ketal (**15c**, 93 mg) was reduced with LAH (240 mg) for 2 hr. Workup and preparative TLC (AB80,8X) of the residue (92 mg) gave two fractions. Fraction one afforded 20 mg of crystalline 16$\beta$,21-*dihydroxyeupha*-7,24-*dien*-3-*one ketal* (**16c**), identical with the product prepared from the free ketone **16**, according to mmp, TLC, IR and NMR.

Fraction two (35 mg) was amorphous but homogeneous by TLC, and was shown to be identical with **18c**, according to TLC, IR and NMR comparisons.

16$\alpha$,21-*Dihydroxyeupha*-7,24-*dien*-3-*one* (**18**). A soln of **18c** (225 mg) in 90% HOAc (25 ml) was heated on a hot plate for 20 min. After removal of solvent, chromatography on Florisil eluted with light petroleum-ether (3:1) afforded a homogeneous product **18** (170 mg) as indicated by TLC; amorphous; $[\alpha]_D$ −39° (*c*, 11); IR($CS_2$) 2·98 (broad, OH); 5·82 $\mu$ (6-ring C=O); NMR: $\tau$8·98, 8·97, 8·95 (6), 8·88 (s, 15, 5 Me); 8·37, 8·30 [bs, 6, >C=C($CH_3$)$_2$]; 6·17 (m, 2, >CHC$\underline{H}_2$OH); 5·43 (m, 1, >C$\underline{H}$OH); 4·85, 4·62 (m, 2, 2 >C=C$\underline{H}$—).

Acetylation of **18** and preparative TLC (AB80, 2X) gave 16$\alpha$,21-*dihydroxyeupha*-7,24-*dien*-3-*one diacetate* (**18a**); amorphous; $[\alpha]_D$ −87° (*c*, 1·0); IR($CS_2$) 5·73, 8·08 (—OAc); 5·84 $\mu$ (6-ring C=O); NMR: $\tau$8·98, 8·95 (9), 8·88 (s, 15, 5 Me); 8·37, 8·28 [bs, 6, >C=C($CH_3$)$_2$]; 7·97, 7·95 (s, 6, 2 —OAc); 5·93 (m, 2, >CHC$\underline{H}_2$OAc); 5·0–4·5 (m, 3, >C$\underline{H}$OAc, >C=C$\underline{H}$—, >C=C$\underline{H}$—).

16$\beta$,21-*Dihydroxyeupha*-7,24-*dien*-3-*one* 3-*ketal*,21-*trityl ether* (**16e**)

The diol ketal **16c** (30 mg) was heated under reflux with trityl bromide (50 mg) in 3 ml of pyridine for 8 hr. The mixture was poured into ice water, acidified with 3% HCl, and extracted with ether. The residue from the ether soln was chromatographed over a Florisil column. Unchanged trityl bromide was eluted in the fractions eluted by light petroleum-ether (15:1); the mono-trityl ether **16e** (11 mg) in the light petroleum-ether (10:1) fractions; and unchanged diol ketal **16c** in the ether fractions. The mono-ether ketal **16e**, an oil, was homogeneous by TLC; its IR spectrum had OH absorption (2·8 $\mu$) and the characteristic trityl group bands at 12·85, 13·0, 13·37 and 14·1 $\mu$ (s); NMR: $\tau$9·22, 9·20, 9·00, 8·80, 8·75 (s, 15, 5 Me), 6·10 (s, 4, —$OCH_2CH_2O$—), 2·75 (m, 18, aromatic).

16$\alpha$,21-*Dihydroxyeupha*-7,24-*dien*-3-*one* 3-*ketal*,21-*trityl ether* (**18e**)

The diol ketal **18c** (25 mg) in pyridine soln was heated with trityl bromide (35 mg) and processed as for the 16$\beta$,21-compound above. The monotrityl ether **18e** was obtained similarly as a homogeneous oil by column chromatography. Its IR spectrum resembled that of **16e**, having the same bands characteristic of OH and trityl groups. However, the NMR spectrum differed appreciably from that of **16e**, just as the spectra of the two free diols **16c** and **18c** differ considerably. NMR: $\tau$9·23, 9·18, 9·10, 8·98, 8·75 (s, 15, 5 Me); 6·07 (s, 4, —$OCH_2CH_2O$—); 2·75 (m, 18, aromatic).

21-*Hydroxyeupha*-7,24-*dien*-3,16-*dione* 3-*ketal* (**19**)

1. The diol mono-trityl ether ketal **16c** was oxidized by Jones reagent in a brief reaction (1 min). Workup and preparative TLC yielded a ketone, an oil; IR($CS_2$): no absorption in OH region; 5·55 (5-ring C=O), 8·9, 9·6 (ketal); 12·82, 13·0, 13·55, 14·15 (s)$\mu$ (trityl); NMR: $\tau$9·18, 9·15, 9·0, 8·87, 8·77, (s, 15, 5 Me), 6·08 (s, 4, —$OCH_2CH_2O$—), 2·75 (m, 18, aromatic).

2. Diol mono-trityl ether ketal **18e** was oxidized in the same way. The product processed similarly was shown to be identical with the ketone described in part I, by IR and NMR comparisons.

16$\alpha$,21-*Epoxyeupha*-7,24-*dien*-3-*one* (**24**). To 16$\alpha$,21-dihydroxyeupha-7,24-dien-3-one (**18**, 68 mg) in pyridine (3 ml) was added MsCl (5 drops). Workup as for **1b** gave starting material **18** (25 mg) and the 16$\alpha$,21 ether **24** (36 mg) as long needles (from MeOH); m.p. 133·0–135·0°; $[\alpha]_D$ −19° (*c*, 1·3); IR($CS_2$) 9·0, 9·25, 9·42, 10·2 $\mu$ (ether); NMR: $\tau$9·03, 8·98, 8·95, 8·94, 8·88 (s, 15, 5 Me); 8·37, 8·28 [bs, 6, >C=C($CH_3$)$_2$]; 6·65 (m, 1, >CHO—); 5·58 (m, 2, >C$\underline{H}$C$\underline{H}_2$O—); 4·87, 4·67 (m, 2, 2, >C=C$\underline{H}$—). (Found: C, 81·77; H, 10·81. Calcd for $C_{30}H_{46}O_2$: C, 82·14; H, 10·57%.)

16$\alpha$,21-*Epoxyeupha*-7,24-*dien*-3-*one ethylene ketal* (**24c**). To a soln of **18c** (44 mg) in pyridine (3 ml) was added MsCl (2 drops). The mixture stood at room temp for 30 min. After workup as for **1b**, and separation by preparative TLC (AB80, 2X) gave the starting diol ketal **18c** (15 mg) and the product **24c** (18 mg) as plates (from MeOH); m.p. 122·5–123·5°; $[\alpha]_D$ +35° (*c*, 1·1); IR($CS_2$) no OH and mesylate bands; 8·3, 8·95, 9·24, 9·55, 9·8 $\mu$ (ketal and ether) NMR: $\tau$9·21, 9·18, 9·03, 8·99 (6) (s, 15, 5 Me); 8·38, 8·30 [bs, 6, >C=C$\underline{M}e_2$]; 6·65 (m, 1, >CHO—); 6·03 (s, 4, —$OCH_2CH_2O$—); 5·58 (m, 2, >C$\underline{H}$C$\underline{H}_2$O—); 4·87, 4·73 (m, 2, 2 >C=C$\underline{H}$—). (Found: C, 79·24; H, 10·28; $M^+$, 482. Calcd for $C_{32}H_{50}O_3$ (482·72): C, 79·62; H, 10·44%.)

*Kulolactone* (**3**) was further purified by preparative TLC; amorphous; $[\alpha]_D$ −42° (*c*, 1·9); ORD (*c*, 0·21; MeOH): $[\phi]_{600}$ −131°; $[\phi]_{589}$ −145°; $[\phi]_{288}$ −1,580°; $[\phi]_{262}$ −920°; $[\phi]_{230}$ −5,050°; $[\phi]_{225}$ −3,940°; IR($CS_2$) 2·7 (—OH); and 5·58, 10·47 $\mu$ ($\gamma$-lactone); NMR: $\tau$9·2, 9·05, 9·02 (6), 8·77 (s, 15, 5 Me); 8·37, 8·27 [bs, 6, >C=C$\underline{M}e_2$]; 6·53 (m, 1, $w/2$ = 7 Hz, >C$\underline{H}$OH), 5·85 (m, 1, $w/2$ = 30 Hz, >CHOOC—); 4·87, 4·68 (m, 2, 2 >C=C$\underline{H}$—). (Found: C, 79·01; H, 9·95. Calcd for $C_{30}H_{46}O_3$: C, 79·25; H, 10·20%.)

*Kulolactone acetate* (**3a**) was prepared and separated in rosettes of needles from MeOH; m.p. 164–166·8°; $[\alpha]_D$ −47·5° (*c*, 2·0); IR($CS_2$) 5·74, 8·04 $\mu$ (—OAc); NMR: $\tau$9·19, 9·13, 9·02, 8·99, 8·77 (s, 15, 5 Me); 8·35, 8·27 [bs, 6, >C=C$\underline{M}e_2$]; 7·92 (s, 3, —OAc); 5·83 (m, 1, $w/2$ = 31 Hz, >CHOOC—); 5·30 (m, 1, $w/2$ = 8 Hz, >CHOAc), 4·87, 4·70 (m, 2, >C=C$\underline{H}$—). (Found: C, 77·98; H, 9·68. Calcd for $C_{32}H_{48}O_4$: C, 77·38; H, 9·74%.)

Kulolactone when briefly (1 min) oxidized by Jones reagent, yielded kulactone, as evidenced by IR, TLC, and GLC comparisons.

3-*Epi*-16-*epikulolactone* (**26**) was prepared by the reduction of **17** (34 mg) with $NaBH_4$. Workup gave the product as rosettes of needles (from MeOH); m.p. 238·4–239·4°; $[\alpha]_D$ +30° (*c*, 1·2); IR($CHCl_3$) 2·71 (OH); 5·66, 8·48 $\mu$ ($\gamma$-lactone); NMR: $\tau$9·22, 9·12, 9·07, 9·00 (6), (s, 15, 5 Me); 8·35, 8·28 [bs, 6, >C=C$\underline{M}e_2$]; 6·72 (m, 1, >C$\underline{H}$OH); 4·98 (m, 1, >CHOOC—); 4·87, 4·65 (m, 2, 2 >C=C$\underline{H}$—). (Found: C, 79·12; H, 10·33. Calcd for $C_{30}H_{46}O_3$: C, 79·25; H, 10·20%.)

*Saponification experiments**

1. Methyl kulonate **4** (10 mg) was heated on a steam bath in 10% methanolic KOH (2 ml), and the reaction was monitored by TLC. The ester was completely saponified in less than 1 hr.

2. Methyl 16-dehydrokulonate **15** under similar conditions was saponified completely within 10 min.

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*In 5% methanolic KOH methyl maecherate, a γ-keto tertiary carboxylic ester was saponified in 17 hr; in 10% solution methyl maechinerate, the corresponding γ-hydroxy ester (hydroxy group cis to carbomethoxy) was saponified in 4 hr.[20]

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