position, since liver lipids and DNA were reduced 12 and 18%, respectively, whereas liver glycogen was elevated 18%. Incorporation of fatty acids into complex lipids appears to be blocked by 7. A similar phenomenon was observed with the bis(β-phenethyl) ketone derivatives.[8]

The fact that the vas deferens, epididymis, vesicular glands, and testes had not undergone atrophy after administration of compound 7 along with a negative uterotropic activity indicated that this compound was not estrogenic at this dose. Furthermore at 50 mg/kg compound 4 possesses no antifertility activity in mice. These characteristics differ from the bis(β-phenethyl) ketone derivatives. Furthermore, the propanone series was not toxic. Small doses (10 mg/kg/day) are adequate to reduce the serum cholesterol in rats significantly compared to clofibrate. The maximum pharmacological effects of these agents require a longer length of time to appear, i.e., 10–14 days compared to the bis(β-phenylethyl ketone) series which requires only 48 hr.[8]

Acknowledgment. We express our sincere appreciation for the interest and encouragement offered by Dr. Robert G. Lamb and for the technical assistance of Charles R. Fenske and Bonnie Whitehead. This investigation was supported by Research Grant HL16464-02 from the Division of Heart and Vascular Diseases, National Heart and Lung Institute, National Institutes of Health.

References and Notes

(1) Predoctoral trainee supported by Public Health Service Training Grant 5T01-GM01770 from the National Institute of General Medical Sciences, National Institutes of Health.
(2) G. L. Carlson, I. H. Hall, G. S. Abernety, and C. Piantadosi, *J. Med. Chem.*, 17, 154 (1974); G. L. Carlson, I. H. Hall, and C. Piantadosi, *ibid.*, 18, 1024 (1975).
(3) K. E. Pfitzner and J. G. Moffatt, *J. Am. Chem. Soc.*, 87, 5661 (1965).
(4) J. Hill, *J. Chem. Soc. C*, 462 (1970).
(5) S. Razniak, Thesis, University Microfilms, Ann Arbor, Mich., 1960, pp 2–3.
(6) C. R. Conard and M. A. Dolliver, "Organic Syntheses", Collect. Vol. II, Wiley, New York, N.Y., 1943, p 167.
(7) A. T. Ness, J. V. Pastewka, and A. C. Peacock, *Clin. Chem. Acta*, 10, 237 (1964).
(8) C. Piantadosi, I. H. Hall, J. L. Irvine, and G. L. Carlson, *J. Med. Chem.*, 16, 770 (1973).
(9) C. W. Emmens, *Methods Horm. Res.*, 2, 61 (1970).
(10) I. H. Hall, G. L. Carlson, G. S. Abernethy, and C. Piantadosi, *J. Med. Chem.*, 17, 1253 (1974).
(11) J. T. Litchfield, Jr., and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, 96, 99 (1949).
(12) I. H. Hall, R. G. Lamb, M. H. H. Mar, G. L. Carlson, and C. Piantadosi, *J. Pharm. Sci.*, 64, 235 (1975).
(13) S. Shibko, P. Koivistoinen, C. A. Tratnyek, A. R. Newhall, and L. Friedman, *Anal. Biochem.*, 19, 514 (1967).
(14) G. W. Snedecor, "Statistical Methods", Iowa State College Press, Ames, Iowa, 1956, p 91.

Novel Pyrazolo, Isoxazolo, and Thiazolo Steroidal Systems and Model Analogs Containing Dimethoxylaryl (or Dihydroxylaryl) Groups and Derivatives. Synthesis, Spectral Properties, and Biological Activity

M. M. Hashem, K. D. Berlin,*

Department of Chemistry

R. W. Chesnut, and N. N. Durham*

Department of Microbiology, Oklahoma State University, Stillwater, Oklahoma 74074. *Received June 9, 1975*

The total syntheses of a series of vicinal-substituted dimethoxy and dihydroxy heterosteroids of the equilenin type and model analogs are described. A novel class of pyrazolo steroidal *N*-glucosides has also been synthesized. Compounds prepared were screened in vitro for growth inhibition of different microorganisms. Of these, 1-α-D-glucopyranosyl-4,5-dihydro-7-methoxy-1*H*-benz[*g*]indazole tetraacetate (13) was quite active. For example, *N*-glucoside 13 inhibited the growth of *Bacillus subtilis*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, and KB cells at moderate concentrations.

The biological activity of heterosteroids[1] with methoxyarene functions as ring A has been evaluated[2] in only a relatively small number of cases.[3] Preliminary results from our laboratory indicated that equilenin-type azasteroids may have bactericidal or bacteriostatic properties as well as ability to potentiate the action of certain drugs.[4] For example, in combination (1:1 molar ratio) with actinomycin D, one of the azasteroids exhibited enhanced antibacterial activity.[5] A working hypothesis was set forth that the observed potentiation may have arisen as a result of formation of a molecular complex between the azasteroid and the anticancer agent.[5,6] NMR (in D_2O), uv, and fluorescence spectroscopic studies supported the idea of such complexation.[5,6]

In continuation of our work in this area, a series of selected heterosteroids,[2] and related model systems, has been synthesized and is described in this paper. An equilenin-type skeleton in the newly synthesized heterosteroids was maintained, with the modification of ring A being substituted with two methoxyl and/or two hydroxyl functionalities. One objective of this work was to determine the biological activity dependence upon the polar nature of end groups in A ring and the small heterocyclic ring. Thus, it was proposed to construct several heterocyclic systems with variations in the five-membered ring (or D ring).

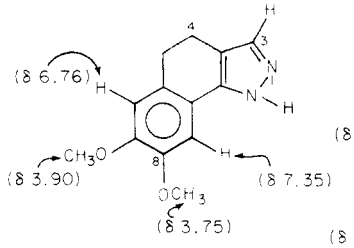
Decreased cell permeability of some heterosteroids and, hence, lack of physiological activity have been attributed to the basic nature of such molecules.[7] Conceivably, heterosteroids with improved water solubility could alter the biological response of a system. This has been partially achieved by addition of a sugar residue to N(1) of the pyrazole ring in a steroidal system. This type of "nucleoside analog" is unknown in literature. Considerable effort has been devoted to the synthesis of nucleosides[8] (and analogs[9]) and application[10,11] thereof in chemotherapy.

Chemistry. A key precursor, 3,4-dihydro-6,7-di-

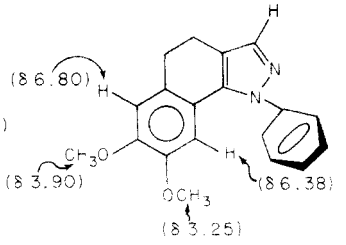
methoxy-1(2*H*)-naphthalenone (1),[12] shown in a general procedure in Scheme I, was involved in the preparation of indazoles **2–13** (Table I), indazol-3-ones **14–18** (Table II), isoxazoles **19–21** (Table III), thiazoles **22–25** (Table IV) and pyrazoles **26–31** (Table V). Indazoles **11–13** were prepared from 6-methoxytetralone. A literature search revealed that no examples of dimethoxy-substituted heterocycles of the classes described herein have been published heretofore.

Synthesis of indazoles **2**, **4**, **6**, **8**, **11**, **26**, and **28** (Tables I and V) was readily accomplished by boiling the required hydroxymethylene ketone precursor (in CH_3OH or CH_3CO_2H) with hydrazine or hydrazine derivatives (Scheme I). Cleavage of the methoxyl groups to form the more water-soluble corresponding diols was achieved by boiling with aqueous 48% HBr.

Proof of structures for all compounds rests upon NMR data along with infrared, mass spectral, and elemental analysis. The NMR spectrum of *N*-aryl substituted pyrazoles **4–8** revealed an upfield shift for the C(9) proton and methoxyl protons on C(8) (in $DCCl_3$). Shifts observed were in the range of 0.3–0.5 ppm. This shielding can be reasonably explained as a result of an out-of-plane positioning of the N(1) benzene ring, as illustrated in structures **2** and **4**, in which H(9) lies above the benzene ring in **4**. This observation supports the previously reported results[13] that hydroxymethylene ketones cyclize with arylhydrazines giving an exclusive product, the N(1) derivative.



2

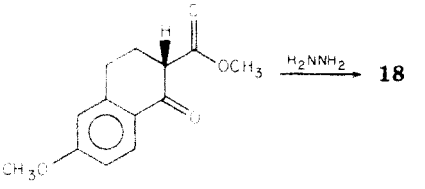


4

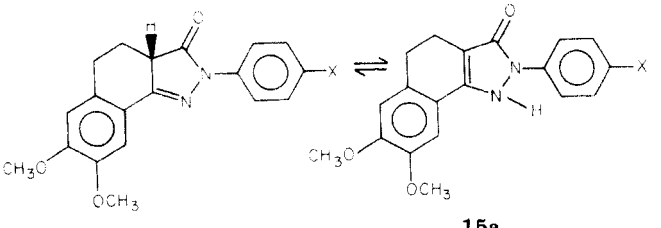
Attachment of a glucose unit to heterosteroids was accomplished de novo (Scheme II). Although N-chloromercuration procedures are known (other methods are also reported[14]), a modified method was used to attach a glucose unit to an N atom of **2**, **32**,[15] and **33**[15] to give **9**, **13**, and **30** (in the acetate form), respectively (Scheme II). The site of glucosidation was at N(1) of the pyrazole ring as evidenced by the nonperturbation of H(3), the signal of the pyrazole ring in NMR spectra taken in different solvents ($DCCl_3$, Me_2SO-d_6, C_5D_5N, and CCl_4). This phenomenon of H(3) perturbation was first observed by Jacquier and co-workers[16] and employed by Alonso and co-workers[17] to establish the site of glycosidation on pyrazole derivatives. The anomeric configuration at C(1) of the sugar molecule was assigned as β since an upfield shift of the H(1) signal of the sugar residue from δ 6.58 [in the starting α-acetobromoglucose (**34**)] to 5.30 (in *N*-glucoside **30**) was observed. Such a diamagnetic shielding effect has been established[18] as a reliable criterion for inversion of configuration from α to β. Also the magnitude of $J_{1',2'}$ coupling of the starting halosugar derivative **34** and $J_{1',2'}$ coupling of the resulting *N*-glucoside supports the β configuration of the anomeric carbon atom. To be specific, the J value for H(1)–H(2) in the halosugar **34** was 2 Hz while it was 7.5 Hz in **13**, 7.0 Hz in **9**, and 8.0 Hz in **30**. Similar arguments supporting the validity of structural assignments have been advanced earlier for somewhat related systems.[17,19] Optical rotation measurements were also consistent with the foregoing conclusions. For example, the value of $[\alpha]^{23}D$ for halosugar **34** was +193.4° (*c* 5.3, $HCCl_3$) while *N*-glucosides **9**, **13**, and **30** had values of −25.5 (*c* 0.9, $HCCl_3$), −86.2 (*c* 2.9, $HCCl_3$), and −41.6° (*c* 0.6, $HCCl_3$), respectively. Large negative $[\alpha]$ values have been reported[20] to indicate inversion of configuration from α to β configuration.

Deblocking the acetate moieties on the glucose residue was carried out using methanolic ammonia. Two free sugar *N*-glucosides **10** and **31** were crystalline. However, the polyol derived from acetate **13** resisted any attempts at crystallization. It was observed that only one *N*-glucoside acetate isomer was obtained in each case from **9**, **13**, or **30**. However, *N*-glucoside acetate **13** was isolated in two different forms, melting at 124–125 and 156–157°, respectively. Both forms were found to have identical properties with regard to NMR, ir, and mass spectra as well as identical R_f (TLC) values in several solvent systems. It has been assumed, therefore, that these forms are merely isomorphic in nature.

Incorporation of a carbonyl function into the heterocyclic ring was accomplished through the preparation of pyrazolones **14–18** (Table II). Two new dimethoxy keto esters **35** and **36** (Scheme III) served as the precursors of those pyrazolones. Cyclization with hydrazines proceeded in excellent yields. Similarly, pyrazolone **18** was synthesized from methyl 1,2,3,4-tetrahydro-6-methoxy-1-oxo-2-naphthoate. Products separated from the cyclization of keto ester **35** with arylhydrazines displayed a carbonyl absorption band in the ir (KBr pellet) spectrum. However, the NMR spectra in $DCCl_3$ did *not* show a signal corresponding to a bridgehead hydrogen. Only a downfield broad singlet was detected (δ 11.10–11.15, one proton). It is suggested, therefore, that tautomerization at *N*(1) occurs with form **15a** (>>**15b**) predominating. Some preliminary observations on tautomerism in compounds related to **18** have been published from our laboratory.[21]

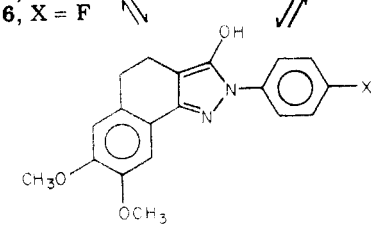


18



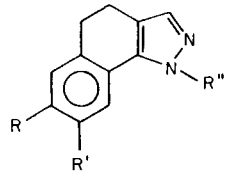
15, X = H
16, X = F

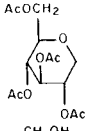
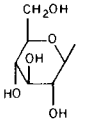
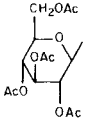
15a



15b

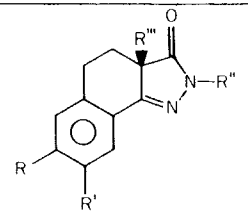
Table I. Substituted 4,5-Dihydro-1*H*-benz[*g*]indazoles



Compd	R	R'	R"	Method	Yield, %	Recrystn solvent	Mp, °C	Formula	Analyses	Growth inhibition: Bacteria[a] *B. sub.*	Growth inhibition: Bacteria[a] *P. flu.*	Growth inhibition: Cell culture,[b] KB cell
					A. 7,8-Dimethoxy (and 7,8-Dihydroxy) Derivatives							
2	OCH_3	OCH_3	H	A	72	*i*-PrOH-H_2O	179.5–180	$C_{13}H_{14}N_2O_2$	C, H, N	+	–	+++
3	OH	OH	H	B	50	H_2O-EtOH	300–302	$C_{11}H_{10}N_2O_2$	C, H, N	+	–	++++
4	OCH_3	OCH_3	C_6H_5	A	85	EtOH	125–126	$C_{19}H_{18}N_2O_2$	C, H, N	+	–	Nt
5	OH	OH	C_6H_5	B	78	EtOH	276–278	$C_{17}H_{14}N_2O_2$	C, H	+	–	++++
6	OCH_3	OCH_3	*p*-C_6H_4-OCH_3	A	52	Heptane	136–137	$C_{20}H_{20}N_2O_3$	C, H, N	+	–	–
7	OH	OH	*p*-C_6H_4-OH	B	74	EtOH-H_2O	295–296	$C_{17}H_{14}N_2O_3$	N	–	–	Nt
8	OCH_3	OCH_3	*p*-SO_2-C_6H_4-CH_3	A	30	EtOH	185–187	$C_{20}H_{20}N_2O_4S$	N	–	–	Nt
9	OCH_3	OCH_3		C	66	$HCCl_3$-petr ether	170–172	$C_{27}H_{32}N_2O_{11}$	N	–	–	Nt
10	OCH_3	OCH_3		C	42	Et_2O	167–168	$C_{19}H_{24}N_2O_7$	N	–	–	Nt
					B. 7-Methoxy (and 7-Hydroxy) Derivatives							
11	OCH_3	H	C_6H_5	A	89	EtOH	105–106	$C_{18}H_{16}N_2O$	N	+	–	Nt
12	OH	H	C_6H_5	B	72	EtOH-H_2O	238–240	$C_{17}H_{14}N_2O$	C, H, N	–	–	Nt
13	OCH_3	H		C	42	Et_2O-petr ether Heptane	125–126 156–157	$C_{26}H_{30}N_2O_{10}$	C, H, N	+	+	++

[a] Microorganisms were grown in glucose minimal medium supplemental with test compound at concentrations of approximately 91 μg/ml and less. The + designates complete growth inhibition at compound concentration limits of 1 and 91 μg/ml; – designates no growth inhibition was observed at the highest concentration limit, 90 μg/ml; Nt, indicates that the compound was not tested. [b] Human tumor cells (KB) were grown in medium 199 plus 10% fetal calf serum supplemented with test compounds at concentrations of approximately 25, 50, and 100 μg/ml. ++++ indicates 50% growth inhibition at a compound concentration of less than 25 μg/ml; +++ indicates 50% growth inhibitions at a compound concentration of 25 μg/ml; + indicates 50% growth inhibition at a compound concentration of 100 μg/ml or greater; – indicates no growth inhibition; Nt indicates that the compound was not tested.

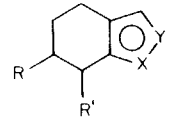
Table II. Substituted 2,3a,4,5-Tetrahydro-7,8-dimethoxy-3*H*-benz[*g*]indazol-3-ones

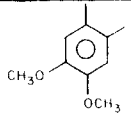
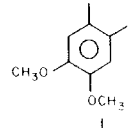
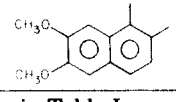


Compd	R	R'	R''	R'''	Method	Yield, %	Recrystn solvent	Mp, °C	Formula	Analyses	Growth inhibition: Bacteria[a] *B. sub.*	Growth inhibition: Bacteria[a] *P. flu.*	Growth inhibition: Cell culture,[b] KB cell
14	OCH_3	OCH_3	H	H	D	91	EtOH	276–277	$C_{13}H_{14}N_2O_3$	N	–	–	Nt
15	OCH_3	OCH_3	C_6H_5	H	D	92	EtOH	200–202	$C_{19}H_{18}N_2O_3$	N	–	–	Nt
16	OCH_3	OCH_3	*p*-C_6H_4F	H	D	65	*i*-PrOH	243–244	$C_{19}H_{17}N_2O_3F$	N, F	–	–	Nt
17	OCH_3	OCH_3	H	CH_3	E	82	EtOH	219–221	$C_{14}H_{16}N_2O_3$	N	+	–	++
18	OCH_3	H	H	H	D	93	EtOH-H_2O	220–222	$C_{12}H_{12}N_2O_2$	N	+	–	++

[a,b] See footnotes in Table I.

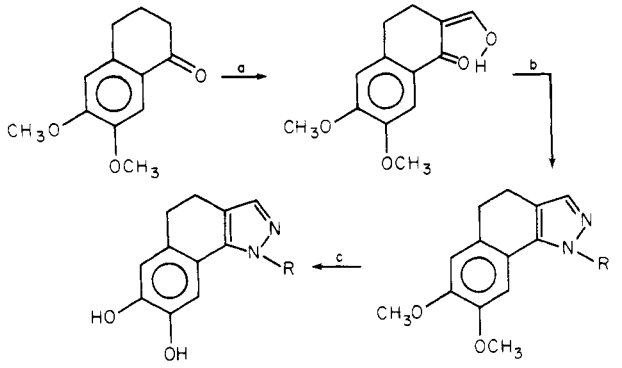
Table III. 4,5-Dihydro-7,8-dimethoxy[2,1-*d*]- and -[1,2-*c*]isoxazoles and 4,5-Dihydro-7,8-dimethoxyphenanthro[2,1-*d*]isoxazole



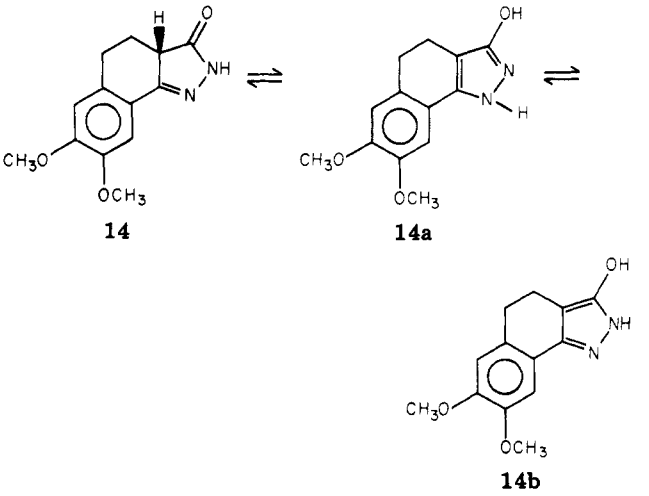
Compd	R + R'	X	Y	Method	Yield, %	Recrystn solvent	Mp, °C	Formula	Analyses	Growth inhibition: Bacteria[a] *B. sub.*	Growth inhibition: Bacteria[a] *P. flu.*	Growth inhibition: Cell culture,[b] KB cell
19		O	N	F	84	Heptane	189–190	$C_{13}H_{13}NO_3$	C, H, N	+	–	–
20		N	O	G	38	Hexane	178–179	$C_{13}H_{13}NO_3$	N	–	–	Nt
21		O	N	F	85	EtOH	194–195	$C_{17}H_{15}NO_3$	C, H	+	–	Nt

[a,b] See footnotes in Table I.

Scheme I



a, $HCO_2C_2H_5$, $NaOCH_3$, C_6H_6
b, H_2NNHR, CH_3CO_2H (or CH_3OH)
c, 48% HBr (under N_2)



14 **14a** **14b**

Cyclizations with *unsubstituted* hydrazines produced pyrazolone **14** with *no* C=O absorption in the ir spectrum of **14a** or **14b** ($FeCl_3$ test was positive). NMR spectra did *not* display a signal correspondent to an angular proton. These types of observations have been noted with only a few other somewhat similar examples in the literature.[22] As expected, and in contrast to pyrazolones **15** and **16**, pyrazolone **17** (with a bridgehead methyl) existed mainly in the keto form as evidenced by both ir and NMR analysis.

A series of 2-substituted thiazoles was also synthesized from 2-bromo-6,7-dimethoxy-1-tetralone (**37**) and thioamides (Scheme IV, Table IV). Cyclization to give the thiazoles proceeded in excellent yields.

Scheme V depicts the preparation of isoxazoles **19–21** (Table III). Isoxazoles **19** and **20** were obtained in two isomeric forms depending on different reaction conditions (see Experimental Section). Some interesting NMR data were revealed for the isoxazoles. Small differences in the chemical shift positions of H(3) of the isoxazole ring have been recorded[23] to distinguish between isomeric isoxazoles. Another feature has been observed in our cases. The proton located on C(9) observed at δ 7.45 in the [1,2-*c*] isomer apparently experienced a virtual long-range shielding and was shifted to δ 7.20 in the [2,1-*d*] isomer. This can probably be attributed to the "sandwiching" of H(9) in close proximity to the two lone pairs on the oxygen atom in the [2,1-*d*] isomer. However, the lone pair on the nitrogen atom in the [1,2-*d*] isomer is likely in the plane

Scheme II

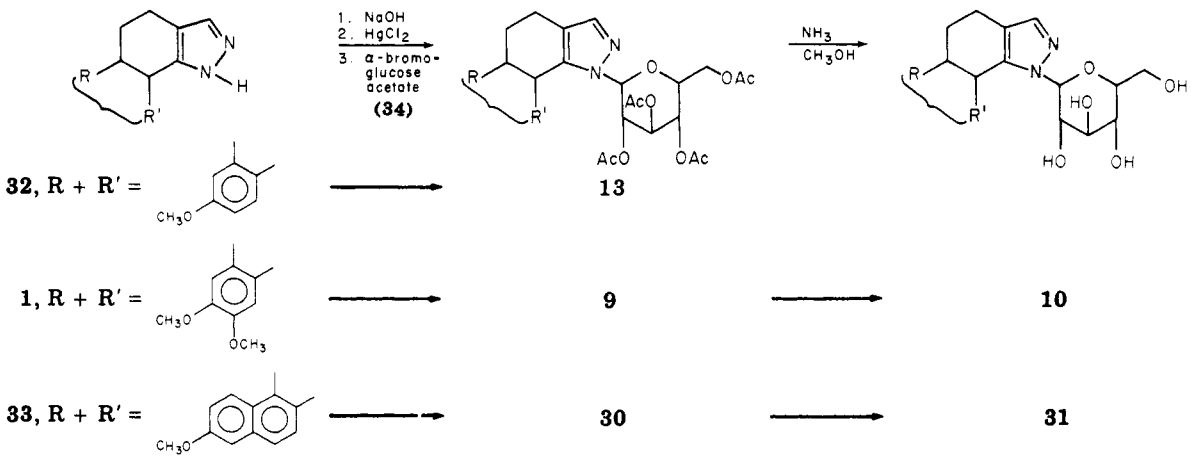
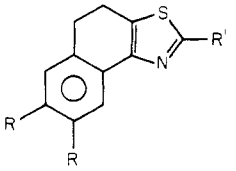


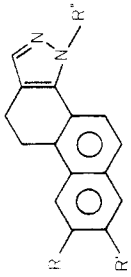
Table IV. 2-Substituted 4,5-Dihydro-7,8-dimethoxy[1,2-*d*]thiazoles (Method H)

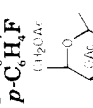


Compd	R	R'	Yield, %	Recrystn solvent	Mp, °C	Formula	Analyses	Growth inhibition: Bacteria[a] *B. sub.*	Growth inhibition: Bacteria[a] *P. flu.*	Growth inhibition: Cell culture,[b] KB cell
22	OCH_3	NH_2	65	EtOH	235-237	$C_{13}H_{14}N_2O_2S$	N	+	–	++
23	OCH_3	NHC_6H_5	90	EtOH	180-181	$C_{19}H_{18}N_2O_2S$	N, S	+	–	++
24	OCH_3	$NHCH_2CH=CH_2$	95	EtOH-H_2O	88-90	$C_{16}H_{18}N_2O_2S$	N, S	+	–	+++
25	OCH_3	CH_3	88	EtOH-H_2O	140-141	$C_{14}H_{15}NO_2S$	C, H, N, S	–	–	Nt

[a,b] See footnotes in Table I.

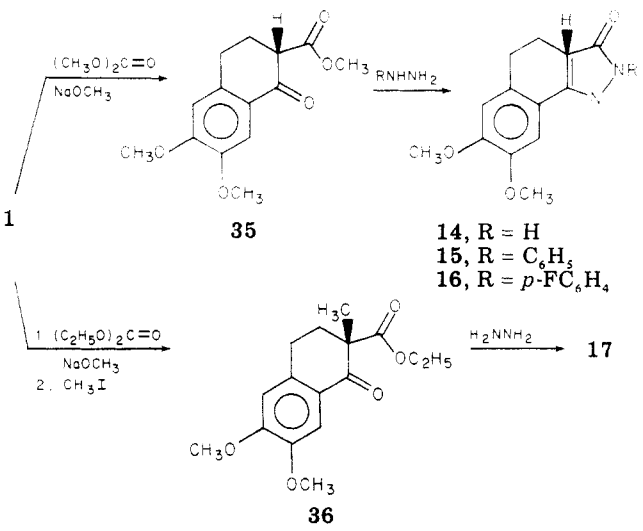
Table V. 10,11-Dihydro-7,8-dimethoxy- (and 7,8-dihydroxy-) phenanthro[1,2-*c*]pyrazoles



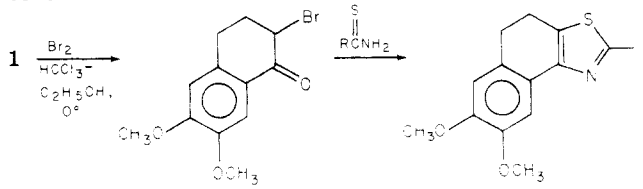
Compd	R	R′	R″	Method	Yield, %	Recrystn solvent	Mp, °C	Formula	Analyses	Growth inhibition: Bacteria[a] *B. sub.*	Bacteria[a] *P. flu.*	Cell culture,[b] KB cell
26	OCH_3	OCH_3	H	A	98	THF–EtOH	277–277.5	$C_{17}H_{16}N_2O_2$	C, H, N	+	–	–
27	OH	OH	H	B	74	H_2O–EtOH	324–236	$C_{15}H_{12}N_2O_2$	C, H, N	–	–	Nt
28	OCH_3	OCH_3	*p*-C_6H_4F	A	64	*i*-PrOH	195–197	$C_{23}H_{14}N_2O_2F$	N, F	–	–	Nt
29	OH	OH	*p*-C_6H_4F	B	51	Acetone–hexane	256–258	$C_{21}H_{15}N_2O_2F$	N, F	+	–	++++
30	H	OCH_3		C	52	$HCCl_3$–petr ether	116–117	$C_{30}H_{32}N_2O_{10}$	N	++	–	++++
31	H	OCH_3		C	14	MeOH–Et_2O	210–212	$C_{22}H_{24}N_2O_6$	C, H, N	–	–	Nt

[a,b] See footnotes in Table I.

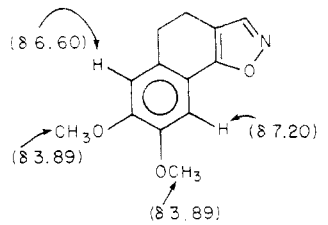
Scheme III



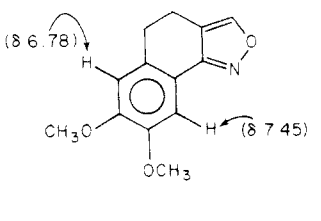
Scheme IV



of the system and the proton on C(9) experiences a shielding effect.



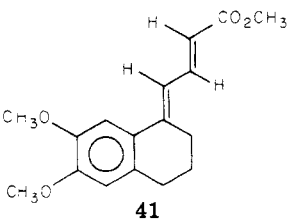
[2,1-*d*] isomer **19**



[1,2-*c*] isomer **20**

The key starting phenanthrone **40** was synthesized in a fair *overall* yield (30% based on **1**) as a precursor for heterosteroids **26–29** and **21** (Table V). Scheme VI describes the steps involved. Somewhat related ketones have been recorded in literature but only in low overall yields, 7–11%.[24]

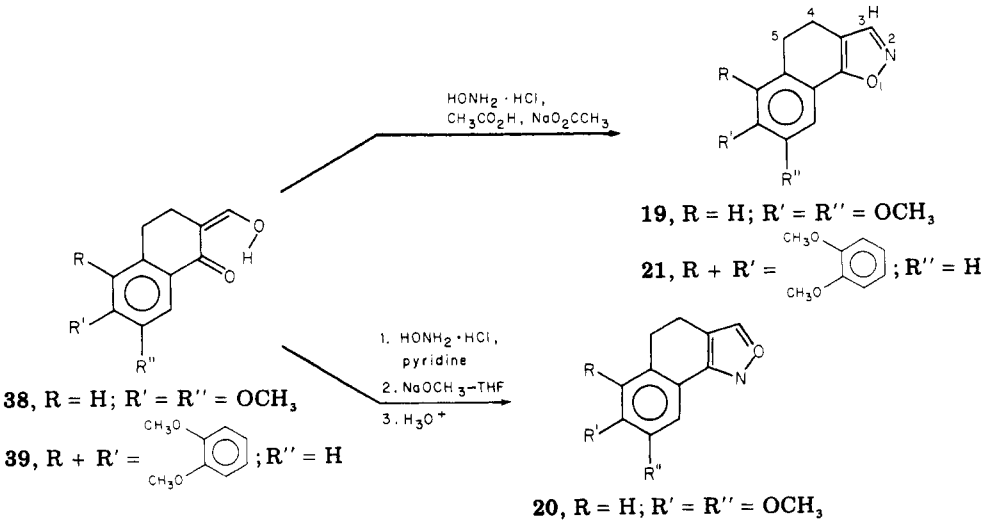
Phenanthrone **40**, previously unknown, was prepared as shown in Scheme VI. The Reformatsky reagent of methyl 4-bromocrotonate and pure zinc was condensed with tetralone **1** to give the conjugated dienic ester **41** (48–51%). NMR spectral data suggest that dienic ester **41** exists in the trans–trans configuration. The J_{H-H} coupling values were almost equal (J = 7 Hz) for protons H_a, H_b, and H_c



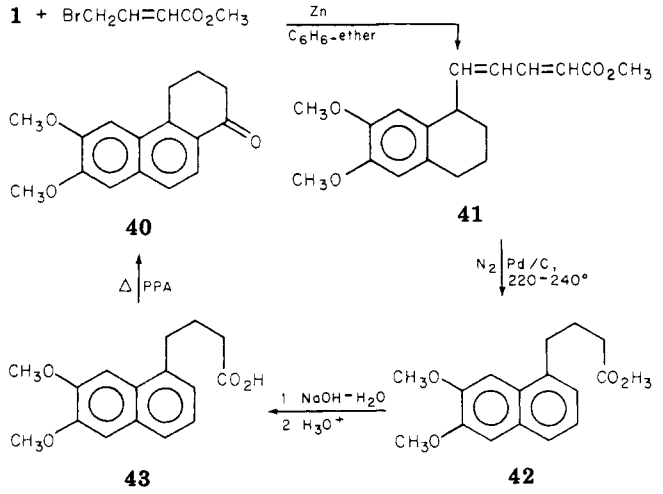
41

and sharp doublets persisted even at a total scan sweep

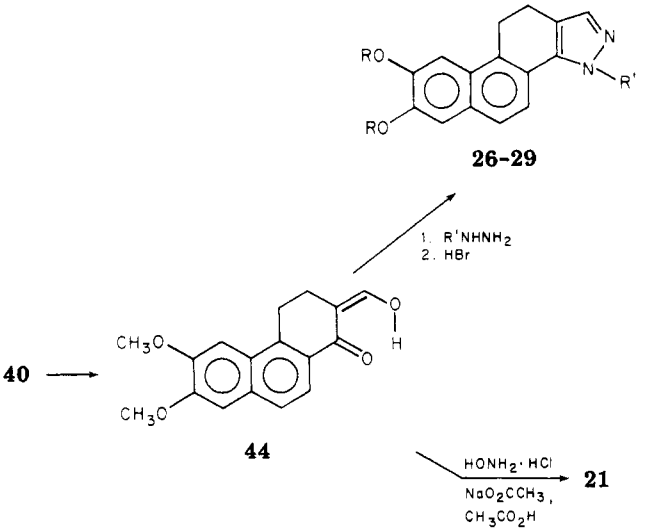
Scheme V



Scheme VI



Scheme VII



width of 25 Hz. If one of the alkenyl protons existed in the cis configuration, more than one J value would have been expected.[25]

Aromatization of dienic ester **41** to the isomerized ester **42** was accomplished by using 10% Pd/C at 220–250°. The resulting ester was saponified, without prior purification, to the butanoic acid precursor **43**. Cyclization of **43** with hot 115% PPA afforded the desired phenanthrone **40** in good yield.

The hydroxymethylene ketone **44**, derived from ketone **40** (93%) by the method used for the model compounds, was utilized to construct the heterocyclic D ring in the steroid system. Thus with $HONH_2$·HCl in glacial acetic acid, isoxazolo steroid **21** was obtained (85%) (Table III). Pyrazolo steroids **26** (98%) and **28** (64%) were prepared from hydroxymethylene **44** by techniques described for the model systems. Phenolic derivatives (74% for **27** and 51% for **29**) of these pyrazolo steroids were readily obtained via treatment of the parent dimethoxy compounds with boiling aqueous 48% HBr (Table V) (see also Scheme VII).

Biological Results. The model systems **2–6**, **11**, **18**, **19**, and **22–24** inhibited growth of *Bacillus subtilis* at 91 μg/ml. Pyrazole **2** completely inhibited the growth of *B. subtilis* and reduced KB cell proliferation by 69% at a concentration of approximately 50 μg/ml. Diol **3** inhibited the growth of *B. subtilis* and completely inhibited KB cell growth at concentrations as low as 12 μg/ml. Administration of a total of 1 mg of diol **3** (1 injection per day ip, 0.2 mg of compound per day for 5 days) to BDF_1 mice bearing L1210 lymphoblastic leukemia produced a T/C ratio of 1.14.

In contrast, the pyrazolo steroid (diol) **27** showed no inhibition of microbial growth. However, dimethoxypyrazolo steroid **26** did inhibit *B. subtilis* (in glucose salts medium) at 91 μg/ml but was ineffective against *Pseudomonas fluorescens* or the cultured KB cells. The presence of a *p*-fluorophenyl group at N(1) of pyrazolo steroid (diol) **29** produced an inhibition of growth of both *B. subtilis* (91 μg/ml) and KB cells (25 μg/ml). KB cells were only 50% inhibited at lower concentrations (down to 10 μg/ml).

Changing one nitrogen atom for an oxygen atom resulted in a sharp change in activity. Model isoxazole **19** inhibited *B. subtilis* growth but only at a concentration of 91 μg/ml; *P. fluorescens* was not affected by **19** up to 91 μg/ml. Isoxazolo steroid **21**, which is an analog of **19**, was also active. Isomeric isoxazole **20** was completely inactive at all concentrations.

Substitution on N(1), in general, reduced the activity of **7–10** (Table I). Replacing other atoms in the five-membered ring as in **14**, **22**, and **24** altered activity significantly. Inhibition of *B. subtilis* at 25 μg/ml by **22** in Me_2SO was observed and KB cells were inhibited completely at 150 μg/ml. The allyl derivative **24** was more effective in inhibition of KB cells at the same concentration as used with **22**. None of the pyrazolones **14–16** showed any significant inhibition of *B. subtilis* with the exceptions

of **17** and **18**. Both latter compounds inhibited *B. subtilis* and KB cells but had no effect on *P. fluorescens* (Table II).

With only one methoxy group on the A ring and substituting N with the tetracetate derivative of glucose gave a rather potent system **13**. Complete inhibition was observed using *B. subtilis* and *P. fluorescens*. Moreover, at 50 μg/ml of **13**, KB cell growth was *terminated*. Interestingly, *N*-glucoside **13** (at 91 μg/ml) inhibited a recent clinical isolate of *Staphylococcus aureus* growing in nutrient broth. *N*-Glucoside **30** was also active against *B. subtilis* and KB cells. In contrast, the *N*-glucosides **10** and **31** with the sugar in the free form (deacetylated) showed no activity nor did the dimethoxy derivative **9**.

Conclusions

It has been shown that the addition of two methoxy functions or two hydroxy functions on the A ring of certain indazoles, isoxazoles, and thiazoles produced compounds with bacteriostatic or bactericidal activity. Substitution at N(1) of the pyrazole by aryl groups also produced inhibition of growth in defined bacterial systems; related pyrazolones, in contrast, were inactive. Structurally similar thiazoles were quite active in general (except **25**).

In an effort to improve water solubility in the general family under discussion, a monomethoxy analog **13** was synthesized and converted to an *N*-glucoside derivative. This compound proved remarkably active against three microorganisms including a *Staph. aureus*. Interestingly, isoxazoles **19** and **21** showed only inhibition of *B. subtilis* and, surprisingly, **20** (isomeric with **19**) was inactive in the primary screens. Thus, replacing one nitrogen with an oxygen atom in the small heterocyclic ring did not change the inhibition observed in the primary screens with but one exception. However, pyrazoles **2**, **3**, and **5** proved highly inhibitory for cell culture growth as did thiazoles **22–24** in contrast to the inactivity of isoxazole **19**. Consequently, the pyrazoles appear to have the most significant activity on a broad scale. Work is in progress to improve the hydrophilicity and lipophilicity of these systems while maintaining the biologically active centers.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Ir spectra were determined with a Beckman IR-5A spectrophotometer on NaCl plates or KBr pellets. NMR spectra were measured with a Varian XL-100 (15) spectrometer (Me_4Si as standard in $DCCl_3$) and chemical shifts are reported in δ (ppm) units: s, singlet; d, doublet; t, triplet; q, quartet; qt, quintet; m, multiplet. The *J* values are in hertz. Mass spectra were determined on a CEC 21-110 B double-focusing mass spectrometer at 70 eV. Elemental analyses were performed by Galbraith Labs., Knoxville, Tenn. All analytical samples gave combustion values for C, H, N, S, and F within ±0.4% of the theoretical values. NMR, ir, and MS data are available upon request.

General Method A. A magnetically stirred mixture of the appropriate hydroxymethylene ketone (0.03 mol) and $RNHNH_2$ (0.1 mol) was warmed (40°, 100 ml of CH_3OH) or boiled (100 ml of CH_3CO_2H) under N_2 for 2–3 hr. The reaction mixture was evaporated to one-fourth the original volume; the concentrate was poured onto ice-cold water (400 ml), and the solid was filtered and recrystallized. Analytical samples of the indazoles or pyrazoles were prepared via sublimation (ca. 5×10^{-4} mm) and/or recrystallization.

General Method B. The methoxyl groups were cleaved by boiling the methoxyindazole (0.02 mol) with 100 ml of 48% HBr for 10–12 hr (under N_2). Upon cooling, usually a pinkish solid was obtained, presumably the hydrobromide of the corresponding indazole. This solid was filtered and redissolved in 10% NaOH solution (20 ml). The resulting dark alkaline solution was filtered from undissolved impurities and neutralized with 6 *N* HCl to afford the crude hydroxyindazole which was filtered, recrystallized, and sublimed (ca. 5×10^{-4} mm).

General Method C. The indazole (a typical case such as **32**) (0.01 mol) was dissolved in 50% boiling ethanol and 0.4 g of NaOH was added. This basic ethanolic solution of the indazole was then added to 0.01 mol of $HgCl_2$ dissolved in 50 ml of 95% ethanol whereby an immediate white product precipitated. The chloromercurio derivative was washed with warm ethanol and cold ether and then dried, giving quantitative yields. Azeotropic distillation from dry xylene (about 100 ml) removed traces of moisture in the chloromercurio derivative. To the cold xylene suspension of the chloromercurio derivative (0.015 mol) was added α-bromoacetoglucose (**34**, 0.02 mol). The heterogeneous mixture was boiled (under N_2) with magnetic stirring for 6–8 hr. Filtration on a sintered glass funnel (medium) removed fine inorganic by-products. Xylene was distilled off and the brown residue was redissolved in $HCCl_3$ and passed through a 4 × 30 cm neutral Al_2O_3 column eluted with $HCCl_3$. Twelve fractions provided the same product (such as **13** from **32**) after work-up which involved evaporating the solvent, trituration with petroleum ether (bp 40–60°), and recrystallization.

General Method D. Keto ester **35** (0.017 mol) was suspended in 100 ml of anhydrous CH_3OH (or glacial CH_3CO_2H) and 0.16 mol of hydrazine (or substituted hydrazine) was added. The reaction mixture was boiled (under N_2) for 3–4 hr. The resulting solution was concentrated (to one-fourth volume) and poured into ice–water (500 ml). Crude indazolones were removed by filtration and dried.

General Method E. (a) Ethyl 1,2,3,4-Tetrahydro-6,7-dimethoxy-2-methyl-1-oxo-2-naphthoate (36). 6,7-Dimethoxy-1-tetralone (**1**, 5.0 g, 0.024 mol) and $NaOCH_3$ (4.2 g, 0.048 mol) were mixed with dry diethyl carbonate (100 g, 0.847 mol), and the mixture was boiled for 2.5 hr under N_2. About 150 ml of CH_3OH was used to dissolve the precipitate formed by cooling the solution. Methyl iodide (9.0 g, 0.063 mol) was added to the resulting solution which was stirred for 18 hr followed by boiling for 1 hr. Neutralization with dilute CH_3CO_2H, removal of organic solvents, and trituration with hot hexane afforded crude keto ester **36**. Recrystallization (95% C_2H_5OH) gave 3.3 g (46%) of ester **36**: mp 74–76°; NMR ($DCCl_3$) δ 1.18 (3 H, t), 1.50 (3 H, s), 2.55–2.95 (4 H, m), 3.95 (3 H, s), 3.96 (3 H, s), 4.15 (2 H, q), 6.60 (1 H, s), 7.50 (1 H, s); ir (KBr) $\nu_{C=O}$ 1650, 1710 cm^{-1}; mass spectra *m/e* calcd for $C_{16}H_{20}O_5$ 292.1259 (M^+), found 292.1311 (M^+).

(b) 2,3a,4,5-Tetrahydro-7,8-dimethoxy-3a-methyl-3*H*-benz[*g*]indazol-3-one (17). Ester **36** (1.2 g, 0.004 mol) was dissolved in 15 ml of CH_3OH and treated with 3 g (0.094 mol) of 95% H_2NNH_2. The reddish-brown solution was stirred (under N_2) and warmed (60°) for 2 hr. Dilution with about 150 ml of cold water afforded indazolone **17** (0.85 g, 82%). Sublimation [200° (0.005 mm)] gave pure **17**: mp 216–218°; NMR ($DCCl_3$) δ 1.35 (3 H, s), 2.0–2.9 (4 H, m), 3.80 (3 H, s), 4.10 (3 H, s), 6.68 (1 H, s), 7.25 (1 H, s), 9.25 (1 H, s); ir (KBr) $\nu_{C=O}$ 1680 cm^{-1}. Anal. ($C_{14}H_{16}N_2O_3$) N.

General Method F. A mixture of $HONH_2 \cdot HCl$ (0.14 mol), $CH_3CO_2Na \cdot 3H_2O$ (0.007 mol) in 5 ml of H_2O, and the required hydroxymethylene ketone (0.086 mol) in 25 ml of glacial CH_3CO_2H was boiled (N_2) for 1–2 hr. Upon cooling, shiny crystals separated out. Filtration afforded crude isoxazoles **19** or **21**. Purification was performed by recrystallization and sublimation.

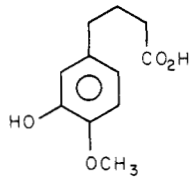
General Method G. A mixture of hydroxymethylene ketone **38** (0.086 mol), $HONH_2 \cdot HCl$ (0.14 mol) in 2 ml of H_2O, and 8 ml of pyridine was boiled for 3 hr. Upon cooling overnight, the dark-green reaction mixture precipitated a brown crystalline product. That product, presumably a mixture of **19** and **20**, was purified by a modification of a method of Guthrie and coworkers.[26] The crude mixture was partitioned between $CH_3CO_2C_2H_5$ (200 ml) and 1 *N* HCl (100 ml) solution. The organic layer was washed twice (30 ml of 1 *N* HCl) and once with saturated NaCl solution (30 ml). Solvents were removed and the residue was redissolved in dry THF (100 ml) containing 0.7 g (0.012 mol) of $NaOCH_3$. The resulting reddish solution was stirred (magnetic) at room temperature (1 hr). Washing with saturated NaCl solution (50 ml), with 2% NaOH (20 ml), and with H_2O (100 ml) and drying the organic layer ($MgSO_4$) gave crude **20** after evaporation

of solvent. Recrystallization and sublimation (10^{-3} mm) afforded pure **20** (light yellow).

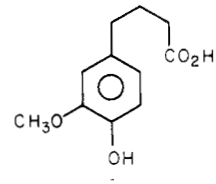
General Method H. To a solution of the bromo ketone **37** (0.007 mol) in 75 ml of $HCCl_3$–C_2H_5OH (1:1) was added 0.04 mol of thiourea. The mixture was boiled (under N_2) with magnetic stirring for 7 hr. Evaporation of the resulting clear solution to dryness gave a white solid, redissolved in 95% C_2H_5OH (220 ml). Cold 2 *N* KOH solution was added dropwise (to ca. pH 10–11) whereby the crude thiazole precipitated and was filtered and dried; it was recrystallized and then sublimed (ca. 5×10^{-4} mm).

3,4-Dihydro-6,7-dimethoxy-1(2*H*)-naphthalenone (1).[12] 4-(3,4-Dimethoxyphenyl)butanoic acid[12,27] (4.0 g, 0.018 mol) was added in small portions with stirring to 50 g of 115% PPA prewarmed to 70–75°. Heating and stirring were continued for 10–15 min. An additional 30 g of PPA was added and the reaction mixture was reheated to 70–75°. Hydrolysis with ice-cold H_2O (ca. 300 ml) afforded solid ketone **1**. It was filtered and washed free of acid (some of $NaHCO_3$). Ketone **1** was dried in air and recrystallized (heptane): yield 3.65 g (83%); mp 98–100° (lit.[28] mp 99–100°).

4-(3,4-Dimethoxyphenyl)butanoic acid[27] was obtained from the catalytic reduction[12] of 4-(3,4-dimethoxyphenyl)-4-oxobutanoic acid (**45**)[28] by hydrogenolysis over 10% Pd/C in glacial CH_3CO_2H. When the Wolff–Kishner method was employed, reduction was accompanied by cleavage of one methoxyl group. The new acid obtained (80%) was identified (NMR, ir, MS, elemental analysis) as 4-(3-hydroxy-4-methoxy)butanoic acid (**46**): mp 87–88° (ether–hexane); NMR ($DCCl_3$) δ 2.0 (2 H, qt), 2.35 (2 H, 5), 2.60 (2 H, t), 3.90 (3 H, s), 5.20–6.20 (2 H, broad), 6.72 (3 H, m); ir (KBr) $\nu_{C=O}$ 1700 cm^{-1}; ν_{OH} 3010, 3045 cm^{-1}. Anal. ($C_{11}H_{14}O_4$) C, H. The isomeric acid **47** is known (mp 120–121°).[29]

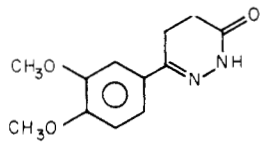


46

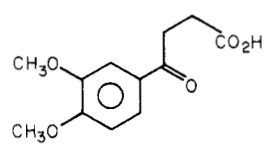


47

A new pyridazinone derivative **48** was synthesized from keto acid **49** by boiling the latter with 1 equiv of 95% H_2NNH_2 in ethanol for 1 hr, concentrating, and cooling to the 6-(3,4-dimethoxy)-4,5-dihydro-3(2*H*)-pyridazinone (**48**): yield 93%; mp 168–170° (C_2H_5OH); NMR ($DCCl_3$) δ 2.62 (2 H, t), 3.00 (2 H, 5),



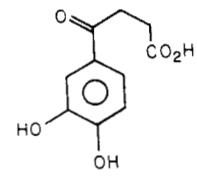
48



49

3.92 (6 H, s), 6.80–7.45 (3 H, m), 9.38 (1 H, s); ir (KBr) $\nu_{C=O}$ 1660 cm^{-1}; ir ν_{NH} 3030 cm^{-1}. Anal. ($C_{12}H_{14}N_2O_3$) N.

An attempt to cleave the methoxyl groups of pyridazinone **48** to the corresponding diol by boiling with aqueous 48% HBr led to the formation of 3-protocatechuoylpropanoic acid (**50**, 16%): mp 190–191° [$(CH_3)_2CHOH$–H_2O]; NMR (Me_2SO-d_6) δ 2.60 (2



50

H, 5), 3.15 (2 H, 5), 6.80–7.50 (3 H, m), 10.00 (3 H, broad); ir (KBr) $\nu_{C=O}$ 1650, 1695 cm^{-1}; ir ν_{OH} 3020, 3030 cm^{-1}. Anal. ($C_{10}H_{10}O_5$) C, H.

3,4-Dihydro-2-(hydroxymethylene)-6,7-dimethoxy-1(2*H*)-naphthalenone (38).[28] Ethyl formate (7.0 g, 0.09 mol) in 50 ml of dry C_6H_6 was added to an ice-cold suspension of $NaOCH_3$ (4.8 g, 0.09 mol) in 75 ml of dry C_6H_6. This mixture was added with stirring to a solution of ketone **1** (9.0 g, 0.044 mol) in 100 ml of dry C_6H_6. After stirring (magnetic) for 1 hr at room temperature (under N_2), the reaction mixture was hydrolyzed with ca. 300 ml of ice-cold H_2O. The organic layer was washed with H_2O, 5% NaOH, and then H_2O. The aqueous phase was extracted with ether (2×100 ml). Acidification (dilute HCl) of the combined alkaline aqueous solutions precipitated yellowish hydroxymethylene ketone **38**: 7–8 g (76%); mp 157–159° (hexane, lit.[29] mp 157–159°); NMR ($DCCl_3$) δ 2.50 (2 H, t), 2.80 (2 H, s), 3.90 (6 H, s), 6.70 (1 H, s), 7.35 (1 H, s), 7.48 (1 H, s), 14.50 (1 H, s); ir (KBr) $\nu_{C=C}$ 1595, 1610 cm^{-1}; ir ν_{OH} 2800 cm^{-1}.

Methyl 1,2,3,4-Tetrahydro-6,7-dimethoxy-1-oxo-2-naphthoate (35). Sodium methoxide (10.0 g, 0.2 mol) was added to dry $(CH_3O)_2C{=}O$ (90.0 g, 1.0 mol) followed by 6,7-dimethoxy-1-tetralone (**1**, 10.0 g, 0.048 mol). The mixture was boiled (4 hr) with stirring under N_2. About 50 ml of CH_3OH was added to dilute the reaction solution which was rendered slightly acidic (cold dilute HCl). Removing the organic solvents gave crude keto ester **35** which was recrystallized (95% C_2H_5OH) to give 11.0 g (92%): mp 140–141°; NMR ($DCCl_3$) δ 2.45 (2 H, m), 2.98 (2 H, t), 3.60 (1 H, t), 3.80 (3 H, s), 3.90 (3 H, s), 3.92 (3 H, s), 6.70 (1 H, s), 7.55 (1 H, s); ir (KBr) $\nu_{C=O}$ 1685, 1710 cm^{-1}. Anal. ($C_{14}H_{16}O_5$) C, H. By a similar procedure methyl 1,2,3,4-tetrahydro-6-methoxy-1-oxo-2-naphthoate (the precursor of **18**) was synthesized (87%): mp 89–90° (C_2H_5OH) (lit.[30] mp 88–89°).

2-Bromo-3,4-dihydro-6,7-dimethoxy-1(2*H*)-naphthalenone (37). By a modification of the method of Wilds,[31] Br_2 (8.8 g, 0.055 mol) was dissolved in 10 ml of $HCCl_3$ and dropped onto a solution of ketone **1** (10.3 g, 0.05 mol) in 100 ml of $HCCl_3$–C_2H_5OH (1:1) at 0°. A yellow precipitate appeared after 1 hr of stirring (magnetic) at room temperature and soon disappeared upon further stirring (3 hr). The reaction mixture was washed (H_2O, saturated $NaHSO_3$, H_2O) and dried (Na_2SO_4). Trituration ($HCCl_3$–ether, 1:4) of the resulting syrup obtained after evaporating the organic solvents gave bromo ketone **37**. Recrystallization ($HCCl_3$–ether) afforded 8.5 g (60%) of **37**: mp 107–108°; NMR ($DCCl_3$) δ 2.5–3.4 (4 H, m), 3.88 (3 H, s), 3.91 (3 H, s), 4.70 (1 H, t), 6.70 (1 H, s), 7.55 (1 H, s); ir (KBr) $\nu_{C=O}$ 1670 cm^{-1}. Anal. ($C_{12}H_{13}O_3Br$) C, H.

Methyl 3,4-Dihydro-6,7-dimethoxy-1(2*H*)-γ-naphthalenecrotonate (41). A solution of 40.0 g (233 mol) of freshly distilled methyl 4-bromocrotonate in anhydrous ether (100 ml) and pure 6,7-dimethoxy-1-tetralone (**1**, 61.8 g, 0.3 mol) in 300 ml of dry C_6H_6 was all added to 75 g (1.15 g-atoms) of previously activated Zn pieces[24] in 100 ml of dry C_6H_6. A crystal of I_2 was added and the reaction was initiated by boiling (1.5 hr) under N_2. The reaction mixture acquired a greenish color and changed to yellow, then to brick-red, and finally to orange during the course of the reaction. An additional 15 g (0.084 mol) of bromo ester was added followed by 25 g (0.382 g-atom) of Zn and a crystal of I_2. Boiling was continued for another 15 hr. The process of addition of reagents and boiling was repeated twice to bring the total amount of reactants used to 150 g of Zn (2.3 g-atoms), 85 g of bromo ester (0.425 mol), and 61.8 g of ketone **1** (0.3 mol). Cold dilute HCl (0.2 *N*, ca. 400 ml) was used to hydrolyze the reaction mixture. The aqueous layer was extracted with C_6H_6 (2×100 ml) and the orange-colored organic solutions were combined, washed with H_2O, and dried ($CaCl_2$). Evaporation of solvents gave dienic ester **41** as an orange solid product. Recrystallization (95% C_2H_5OH) and sublimation [130° (10^{-4} mm)] afforded pure 42 g (49%) of dienic ester **41** (bright yellow): mp 142–143°; NMR ($DCCl_3$) δ 1.25 (2 H, s), 1.90 (2 H, qt), 3.35 (2 H, t), 3.75 (3 H, s), 3.88 (3 H, s), 3.91 (3 H, s), 6.65 (1 H, d), 5.88 (1 H, d), 6.55 (1 H, s), 7.15 (1 H, s), 7194 (1 H, 2 d); ir (KBr) $\nu_{C=O}$ 1695 cm^{-1}. Anal. ($C_{17}H_{20}O_4$) C, H.

6,7-Dimethoxy-1-naphthalenebutanoic Acid (43). A mixture of 12.2 g (0.043 mol) of pure dienic ester **41** and 3.0 g of 10% Pd/C was heated (under CO_2) at 220–250° for 2–3 hr. After cooling (under CO_2), ether (50 ml) was shaken with the reaction mixture and the catalyst was filtered off. Evaporation of ether gave crude methyl 4-(6,7-dimethoxy-1-naphthyl)butanoate (**42**, 10.0 g, 83%). Without further purification, ester **42** was saponified

by boiling (3–4 hr) with 3.5 g of KOH in 30 ml of H_2O and 100 ml of CH_3OH. Acidification (dilute HCl) precipitated crude acid **43**. Recrystallization [$(CH_3)_2CHOH$–H_2O] and sublimation [120° (10^{-4} mm)] yielded 9.1 g (95%) of pure acid **43**: mp 134–135°; NMR ($DCCl_3$) δ 2.15 (2 H, qt), 2.55 (2 H, t), 3.10 (2 H, t), 3.98 (3 H, s), 4.00 (3 H, s), 5.55 (1 H, s), 7.10–7.65 (5 H, m); ir (KBr) $\nu C{=}O$ 1695 cm^{-1}; ir νOH 3035 cm^{-1}. Anal. ($C_{16}H_{18}O_4$) C, H.

3,4-Dihydro-6,7-dimethoxy-1(2*H*)-phenanthrone (40). Acid **43** (20.0 g, 0.73 mol) was added in small portions to stirred 115% PPA (ca. 100 g) prewarmed to 90–100°. Stirring was continued for 15 min followed by the addition of 50 g of PPA; the mixture was reheated to 100° for 10 min. The dark reaction mixture was cooled to room temperature with continuous stirring. Hydrolysis with ca. 800 ml of ice–water precipitated crude phenanthrone **40**. Purification was accomplished by extraction of the dry crude phenanthrone **40** in a Soxhlet apparatus (heptane) for 48 hr. Pure phenanthrone **40** precipitated in the heptane solution as the extraction was continued. Filtration of the heptane suspension and evaporation of the solvent gave a second crop of ketone **40**: total yield 15.4 g (85%); mp 198–200°. Sublimation [150° (10^{-4} mm)] yielded a pure product, mp 210–211° (lit.[32] 215–215.5°). Only the melting point and NMR data are reported for ketone **40** as a by-product[32]: mass spectra ($C_{13}H_{16}O_3$) *m/e* calcd 220.1099 (M^+), found 220.1089 (M^+); ir (KBr) $\nu C{=}O$ 1660 cm^{-1}.

3,4-Dihydro-2-(hydroxymethylene)-6,7-dimethoxy-1(2*H*)-phenanthrone (44). A solution of ketone **40** (8.0 g, 0.03 mol) in dry C_6H_6 was added to a suspension of $NaOCH_3$ (3.0 g, 0.07 mol) in 50 ml of dry C_6H_6. Ethyl formate (12.0 g, 0.14 mol) was then added, and the reaction was initiated by stirring at room temperature (under N_2) for 6 hr. Ice–water (ca. 800 ml) was used to hydrolyze the reaction mixture. The C_6H_6 layer was extracted with H_2O (100 ml) and the aqueous layer was extracted with 5% NaOH (50 ml). Acidification of the combined alkaline aqueous solutions gave a yellowish product, hydroxymethylene ketone **44**. Recrystallization (hexane) and sublimation [120° (10^{-3} mm)] afforded pure **44** (8.0 g, 93%) as a yellow crystalline product: mp 147–148°; NMR ($DCCl_3$) 2.70 (2 H, 5), 3.26 (2 H, 5), 4.08 (6 H, s), 7.10 (1 H, s), 7.30 (1 H, s), 7.60 (1 H, d), 7.89 (1 H, s); ir (KBr) $\nu C{=}O$ 1610 cm^{-1}. Anal. ($C_{17}H_{16}O_4$) C, H.

Biological Testing. Procedures for testing the compounds for biological activity have been described previously.[4b,c,33] Briefly, bacteriological evaluation consisted of suspending a desired concentration of the test compound in water containing 0.1% Me_2SO. The test material was then added to tube cultures of *B. subtilis* or *P. fluorescens* growing on glucose minimal medium[4b,c] to give a maximum final steroid concentration of 91 μg/ml. In one experiment, a recent clinical isolate of *Staph. aureus* was tested growing in tube cultures of nutrient broth (Difco) under similar conditions. The compound's effect on growth was determined by incubating the cultures and periodically measuring their change in absorbance (540 nm) for a period of 24 hr.

Tissue culture, KB cell testing was conducted by the addition of the test compound to 10 × 35 mm Falcon plates containing 300 KB cells in medium 199 plus 10% calf serum.[4c] The cells were incubated for 7 days and stained and the colonies were counted microscopically. Control plate counts were designated as zero inhibition and used as a comparison for the inhibition of KB cell growth by the test compound and as a viability check.[4b]

Preliminary testing against L1210 lymphoblastic leukemia was conducted in 35-day-old female BDF_1 mice (Sprague–Dawley, Madison, Wis.). Animals (ten per test group) were injected with 1×10^6 viable L1210 cells ip on day 0 and administered one injection of test compound (0.2 mg per injection, suspended in saline containing 0.05% carboxymethylcellulose ip) per day on days 1, 2, 3, 4, and 5 (1 mg total injected). Control animals were injected with the saline–carboxymethylcellulose carrier only. The antileukemia activity of the compounds was judged by the prolongation in mean survival time (days) of the test mice compared to the control. The data are expressed as the ratio of mean survival times of the test group to the control (T/C ratio).

Acknowledgment. We gratefully acknowledge partial support of this work by the U.S. Public Health Service, National Cancer Institute, Grant No. CA 14343. We thank Dr. K. Loening (Chemical Abstracts) for aid in naming the compounds found in this paper. We also thank Mr. J. P. Cassidy of FMC Corp., N.Y., for encouragement and generous supplies of 115% PPA. We also gratefully acknowledge preliminary support from the Biomedical Support Grant to the Oklahoma State University.

References and Notes

(1) For a general review of this area, see M. M. Hashem, Ph.D. dissertation submitted in the Oklahoma State University, May 1975.
(2) This work was presented, in part, at the Tetrasectional ACS meeting, Tulsa, Okla., March 15, 1975. See also E. A. Mawdsley, K. D. Berlin, R. W. Chesnut, and N. N. Durham, *J. Med. Chem.*, following paper in this issue.
(3) R. E. Lyle and G. A. Heavner, *J. Org. Chem.*, **40**, 50 (1975); H. O. Huismann, *MTP Int. Rev. Sci., Org. Chem., Ser. One*, **8**, 235 (1973).
(4) (a) J. G. Morgan, K. D. Berlin, N. N. Durham, and R. W. Chesnut, *J. Org. Chem.*, **36**, 1599 (1971); (b) M. L. Higgins, R. W. Chesnut, F. R. Leach, J. G. Morgan, K. D. Berlin, and N. N. Durham, *Steroids*, **19**, 301 (1972); (c) R. W. Chesnut, D. F. Haslam, N. N. Durham, and K. D. Berlin, *Can. J. Biochem.*, **50**, 516 (1972).
(5) R. W. Chesnut, N. N. Durham, E. A. Mawdsley, G. X. Thyvelikakath, and K. D. Berlin, *Biochem. J.*, **143**, 789 (1974).
(6) N. N. Durham, R. W. Chesnut, D. F. Haslam, and K. D. Berlin, *Ann. Okla. Acad. Sci.*, 77 (1974).
(7) M. Martin-Smith and M. F. Sugrue, *J. Pharm. Pharmacol.*, **16**, 596 (1964); S. V. Kessar, M. Singh, V. K. Ahuja, and A. K. Lumb, *J. Chem. Soc. C*, 262 (1971).
(8) W. W. Zorbach, *Synthesis*, 329 (1970); C. A. Dekker and L. Goodman in "The Carbohydrates, Chemistry and Biochemistry", Vol. IIA, 2nd ed, W. Pigman and D. Horton, Ed., Academic Press, New York, N.Y., 1970, Chapter 29.
(9) L. B. Townsend and G. R. Revankar, *Chem. Rev.*, **70**, 389 (1970).
(10) R. W. Sidwell, J. H. Huffman, G. P. Khare, L. B. Allen, J. T. Witkowski, and R. K. Robins, *Science*, **177**, 705 (1972); K-Y. Z. Cheng and C. C. Cheng, *J. Pharm. Sci.*, **59**, 1630 (1970).
(11) J. A. Montgomery, T. P. Johnson, and Y. F. Shealy in "Medicinal Chemistry", Part I, 3rd ed, A. Burger, Ed., Wiley-Interscience, New York, N.Y., 1970, Chapter 28.
(12) T. E. Snider, M. M. Hashem, K. D. Berlin, R. W. Chesnut, and N. N. Durham, *Org. Prep. Proced. Int.*, **5**, 29 (1973).
(13) R. O. Clinton, A. J. Manson, F. W. Stonner, H. C. Neumann, R. G. Christiansen, R. L. Clarke, J. H. Ackerman, D. F. Page, J. W. Dean, W. B. Dickinson, and C. Carabateas, *J. Am. Chem. Soc.*, **83**, 1478 (1961).
(14) J. A. Cleaver, A. B. Foster, and W. G. Overend, *J. Chem. Soc.*, 409 (1959); J. Davoll and G. B. Brown, *J. Am. Chem. Soc.*, **73**, 5781 (1951).
(15) J. G. Morgan, K. D. Berlin, N. N. Durham, and R. W. Chesnut, *J. Heterocycl. Chem.*, **8**, 61 (1971).
(16) J. Elguero, R. Jacquier, and H. C. N. Tien Duc, *Bull. Soc. Chim. Fr.*, 3744 (1966); J. Elguero, A. Fruchier, and R. Jacquier, *ibid.*, 2075 (1966).
(17) G. Alonso, G. Garcia-Munoz, and R. Madronero, *J. Heterocycl. Chem.*, **7**, 1435 (1970).
(18) R. V. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Am. Chem. Soc.*, **79**, 1005 (1957); **80**, 6098 (1958).
(19) I. A. Korbukh, M. N. Preobrazhenkeya, and O. N. Judina, *J. Carbohydr. Nucleosides, Nucleotides*, **1**, 363 (1974).
(20) B. R. Baker, *Chem. Biol. Purines, Ciba Found. Symp., 1956*, 120–130 (1957).
(21) G. X. Thyvelikakath, L. J. Bramlett, T. E. Snider, D. L. Morris, D. F. Haslam, W. D. White, N. Purdie, N. N. Durham, and K. D. Berlin, *J. Heterocycl. Chem.*, **11**, 189 (1974).
(22) H. J. Davies and B. G. Main, *J. Chem. Soc. C*, 327 (1970).
(23) L. Bardou, J. Elquero, and R. Jacquier, *Bull. Soc. Chim. Fr.*, **289**, 297 (1967).

(24) G. Stork, *J. Am. Chem. Soc.*, **69**, 2936 (1947).
(25) N. Muller and P. J. Schultz, *J. Phys. Chem.*, **68**, 2026 (1964); N. Muller *J. Chem. Phys.*, **37**, 2729 (1962); A. A. Brothers-By, *Adv. Magn. Reson.*, **1**, 195 (1965); S. Sternhell, *Rev. Pure Appl. Chem.*, **14**, 15 (1964); R. T. Hobgood, Jr., and J. H. Goldstein, *J. Mol. Spectrosc.*, **12**, 76 (1964).
(26) R. W. Guthrie, A. Borois, J. G. Mullin, F. A. Mennona, and R. W. Kierstead, *J. Med. Chem.*, **16**, 257 (1973).
(27) R. W. Haworth and C. R. Mavin, *J. Chem. Soc.*, 1948 (1932).
(28) K. N. Campbell, A. Schrage, and B. K. Campbell, *J. Org. Chem.*, **15**, 1135 (1950).
(29) F. Gaslini and L. Z. Nahum, *J. Org. Chem.*, **29**, 1177 (1964).
(30) E. Buchta, H. Galster, and F. Luther, *Chem. Ber.*, **82**, 126 (1949); J. Jacques and A. Horeau, *Bull. Soc. Chim. Fr.*, 512 (1950); W. E. Bachmann and D. G. Thomas, *J. Am. Chem. Soc.*, **64**, 94 (1942).
(31) A. L. Wilds, *J. Am. Chem. Soc.*, **64**, 1421 (1942).
(32) S. Imai, *J. Sci. Hiroshima Univ., Ser. A-2*, **33**, 295 (1969); **35**, 161, 171 (1971).
(33) S. R. Holbrook, D. van der Helm, W. Taylor, R. W. Chesnut, N. N. Durham, M. L. Higgins, T. E. Snider, and K. D. Berlin, *Phosphorus*, **6**, 15 (1975).

Synthesis and Structure–Activity Relationships of Heterocyclic Compounds Containing a Trimethoxyarene Function

E. A. Mawdsley, K. D. Berlin,*

Department of Chemistry

R. W. Chesnut, and N. N. Durham

Department of Microbiology, Oklahoma State University, Stillwater, Oklahoma 74074. *Received June 9, 1975*

Pyrazole-, isoxazole-, and pyrazolone-containing systems were prepared from 3,4-dihydro-5,6,7-trimethoxy-1(2*H*)-naphthalenone, 3,4-dihydro-6,7,8-trimethoxy-1(2*H*)-naphthalenone, and 3,4-dihydro-6,7,8-trimethoxy-1(2*H*)-phenanthrone. Primarily, the pyrazoles displayed inhibition of growth in the microbial screens and in tissue culture. Correlation of the heteroatom distances between the oxygen atoms of two methoxy groups and a nitrogen atom in the pyrazole function with the percent plating efficiency on KB cell growth suggests increased inhibition as the $(O^A–N)/(O^B–N)$ ratio deviates from one. No trend was observed in relating the $O^A–N–O^B$ angle and activity for the examples studied.

A large number of polymethoxyarene-substituted compounds are known to be physiologically active.[1-3] A number of physiologically active azasteroids have been reported and several reviews on this subject have been written[4] but very few contain a polymethoxyarene group. As part of our continuing study of the activity of azasteroid systems[5] we selected for study pyrazoles, isoxazoles, and pyrazolones synthesized from 3,4-dihydro-5,6,7-trimethoxy-1(2*H*)-naphthalenone (**1a**),[5] 3,4-dihydro-6,7,8-trimethoxy-1(2*H*)-naphthalenone (**1b**),[6] and 3,4-dihydro-6,7,8-trimethoxy-1(2*H*)-phenanthrone (**2**). This paper reports the synthesis of these compounds and the correlation of heteroatom distances and plating efficiency of KB cells determined for the pyrazole analogs.

Chemistry. Phenanthrone **2** was synthesized from **1a** by initial condensation with methyl 4-bromocrotonate in a Reformatsky reaction. This was followed by dehydration and isomerization to form the naphthalene butyric ester **3** which was saponified in aqueous KOH. The resulting acid **4** was cyclized in the presence of polyphosphoric acid (PPA) to form **2** (Scheme I).

Treatment of **1a**, **1b**, or **2** with ethyl formate in the presence of $NaOCH_3$ gave the corresponding hydroxymethylene derivative **5a**, **5b**, or **6**. Pyrazole derivatives **7a**, **7b**, and **8** were obtained by treatment of the corresponding hydroxymethylene derivative with hydrazine in methanol (Schemes II and III).

The isoxazole derivatives **9a**, **9b**, and **10** were prepared[7] from the corresponding hydroxymethylene compounds for the formation of the [2,3-*d*] isomer. Formation of the α-keto ester **11a** from **1a** was successfully achieved by heating **1a** in anhydrous dimethyl carbonate while treatment of **11a** with hydrazine gave pyrazolone **12a** (Scheme IV).

In one case the α-keto ester **11a** was not isolated but, in the presence of an additional equivalent of base, CH_3I in CH_3OH was added to form **11b**. Likewise, **11c** was prepared from the tetralone **1b** and **13** was prepared from the phenanthrone **2**. Treatment of **11a**, **11b**, or **13** with hydrazine in methanol yielded the corresponding pyrazolones **12a**, **12b**, and **14** (Scheme V).

Biological Results and Discussion. *Bacillus subtilis* W23 (a prototrophic strain) and *Pseudomonas fluorescens* NND were chosen for the microbial screening (Table I).

Scheme I