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POLYHYDROXYNAPHTHOQUINONES

PREPARATION AND HYDROLYSIS OF METHOXYL DERIVATIVES[1a]

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Abstract—The conditions for selective methylation and hydrolysis were studied for a large number of polyhydroxynaphthoquinones. As a result it has become possible to predict the course of these reactions and therefore utilize these derivatives for readier separation and identification of naturally occurring echinoderm pigments.

OUR structural studies of echinoderm pigments frequently pointed to a need for well-defined derivatives of these highly polar polyhydroxynaphthoquinones. Such derivatives would render the compounds less polar and therefore less sensitive to air, more soluble in the common spectral solvents, and more readily separable by chromatography. Among the small number of available choices the methyl ethers seemed to be most promising since they are easily prepared in good yield and since, in the case of hydroxyquinones, the parent compounds can be regenerated without difficulty. Furthermore, a detailed methylation study of the more common echinoid pigments appeared worthwhile as spinochrome E monomethyl ether has already been identified in a holothurian[2] and we have recently encountered two dimethyl ethers of spinochrome E in an asteroid and a monomethyl ether of spinochrome A in an ophiuroid.[3] Our work along these lines has shown that these apparently clear-cut reactions, methylation and demethylation, are not without ambiguity nor without intrinsic interest. The present paper reports some of our observations.

Juglone derivatives

Methylation. OH groups attached to the quinoid portion of a juglone (5-hydroxynaphthoquinone) are more acidic than are hydroxyls attached to the benzenoid part. A small excess of diazomethane therefore effects instantaneous and quantitative methylation of quinoidal hydroxyls. A large excess of diazomethane and longer reaction times (15–30 min) are necessary to methylate normal phenolic hydroxyls on the benzenoid portion of the juglone system. Under these conditions the strongly hydrogen-bonded *peri* OH is not methylated. The results are tabulated in Tables 1 and 2.

An OH at C-3 apparently is more acidic than one at C-2. When 2,3,7-trihydroxy-6-ethyljuglone (**1**) is partially methylated with diazomethane, the principal mono-

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[2] M. Yamaguchi, T. Mukai and T. Tsumaki, *Mem. Fac. Sci., Kyushu Univ. Ser. C.* **4**, 193 (1961).

[3] H. Singh, R. E. Moore and P. J. Scheuer, in preparation.

TABLE 1. RAPID METHYLATION OF HYDROXYJUGLONES WITH DIAZOMETHANE

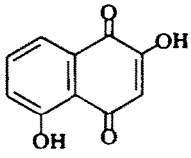
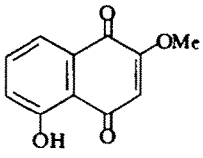
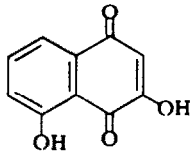
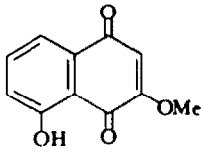
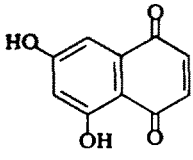
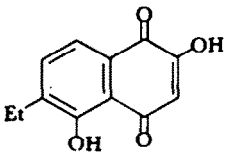
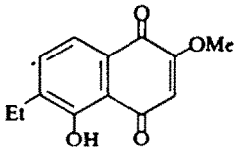
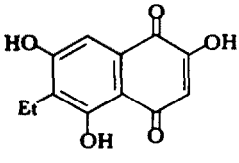
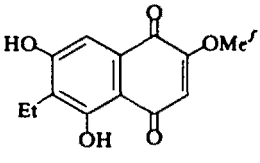
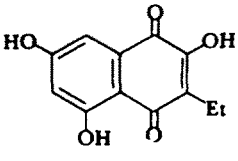
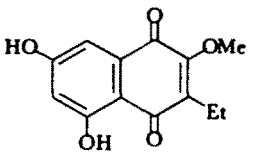
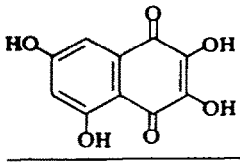
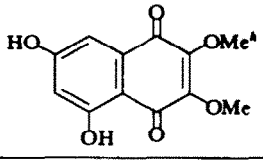
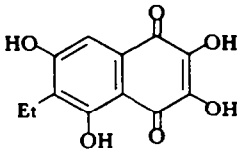
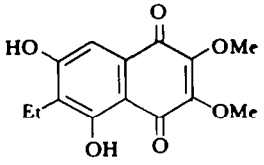
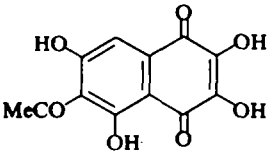
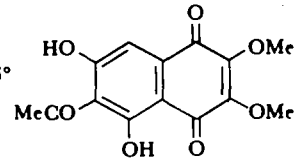
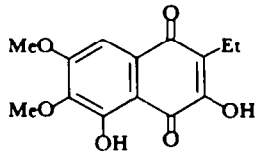
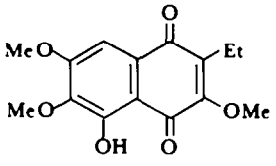
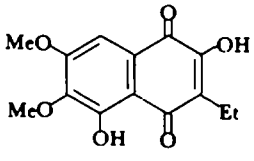
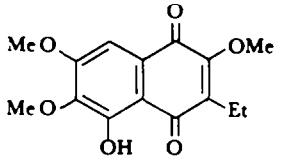
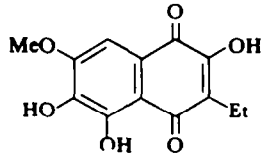
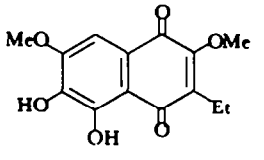
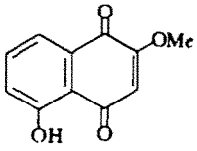
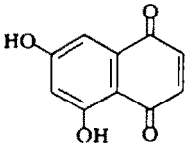
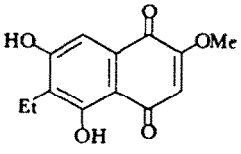
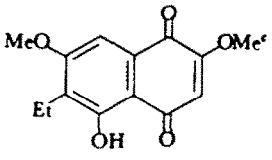
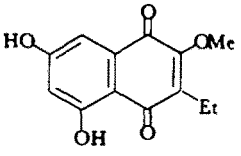
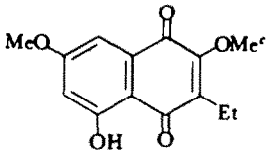
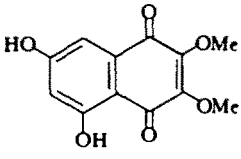
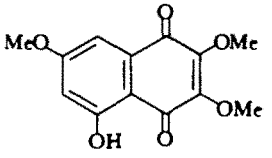
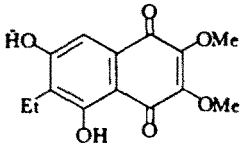
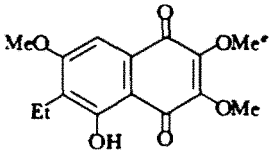
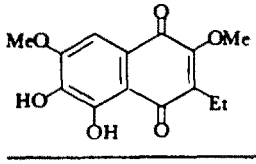
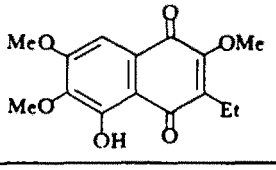
Reactant			Product		
Structure	Relative R_f[a]	M.p.	Structure	Relative R_f[a]	M.p.
	0·430	215–218° (dec)[b]		0·452	159–160°
	0·286	216–218° (dec)[c]		0·381	222–222·5° with subl
	0·109	163–167° (dec)[d]	No reaction		
	0·618	219–220°[e]			137–138°[f]
	0·048	237° (dec)[g]		0·076	252–254°
	0·082	219–220°[e]		0·139	194·5–195°
	0·001	>300°[g]		0·037	204–205°

TABLE 1—*cont.*

Reactant			Product		
Structure	Relative R_f[a]	M.p.	Structure	Relative R_f[a]	M.p.
	0·008	265–269° (dec and subl)[f]		0·065	207–209°
	0·050	245–255° (subl)[f]		0·462	134–135°[d]
	0·385	185–187°		0·480	141–142°
	0·429	165·5°		0·560	105°
	0·218	232–233°		0·278	160°

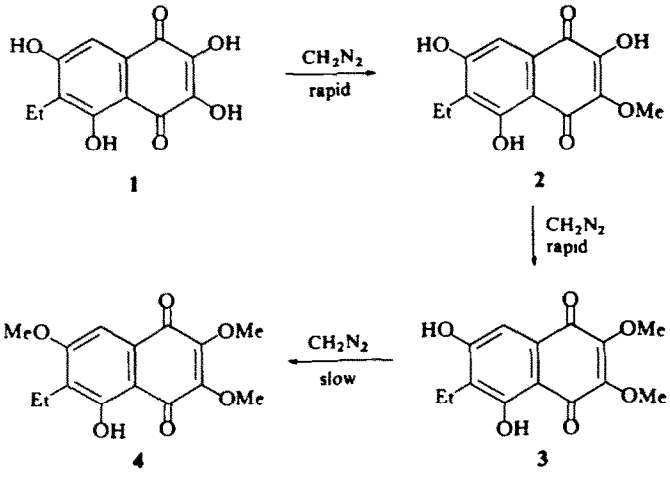
[a] On thin-layer plate of acid-washed, deactivated silica gel with benzene; referred to naphthazarin (R_f = 1·000). The R_f-values are not reproducible and will vary appreciably depending on the characteristics of the plate. These R_f-values were determined simultaneously and can be compared with those reported in other Tables in this paper and other related papers from this laboratory. [b] Reported m.p. 220° (dec) [R. H. Thomson, *J. Org. Chem.* 13, 870 (1948)]. [c] Reported m.p. 218–220° (dec) [R. H. Thomson, *Ibid.* 13, 870 (1948)]. [d] Reported m.p. 165–170° (dec) [J. F. Garden and R. H. Thomson, *J. Chem. Soc.* 2483 (1957)]. [e] R. E. Moore, H. Singh, C. W. J. Chang and P. J. Scheuer, *J. Org. Chem* 31, 3638 (1966). [f] Found: C, 62·70; H, 5·05. $C_{13}H_{12}O_5$ requires: C, 62·90; H, 4·87%. [g] Reported m.p. 325–330° [J. Gough and M. F. Sutherland, *Tetrahedron Letters 269* (1964)]. [h] Found: C, 57·85; H, 4·49. $C_{12}H_{10}O_6$ requires: C, 57·60; H, 4·03%. [i] R. E. Moore, H. Singh and P. J. Scheuer, *J. Org. Chem.* 31, 3645 (1966). [j] Found: C, 60·17; H, 5·03. $C_{14}H_{14}O_6$ requires: C, 60·43; H, 5·07%.

TABLE 2. SLOW METHYLATION OF HYDROXYJUGLONES WITH DIAZOMETHANE

Reactant			Product		
Structure	Relative R_f[a]	M.p.	Structure	Relative R_f[a]	M.p.
	0·452	159–160°	No reaction		
	0·109	163–167° (dec)	mixture[b]		
	0·076	252–254°		0·510	189–189·5°
	0·139	194·5–195°		1·105	69–70°
	0·037	204–205°		0·381	113–114°[d]
	0·065	207–209°		0·462	113–114°
	0·278	160°		0·560	105°

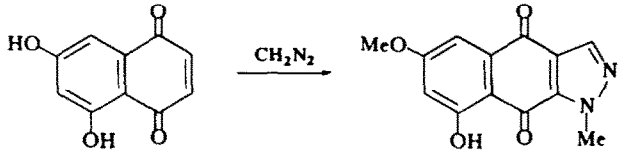
[a] See footnote *a* in Table 1. [b] Besides methylation of the C-7 OH, addition reactions of diazomethane to the quinoid ring occur. [c] See footnote *e* in Table 1. [d] Reported m.p. 112° [J. Gough and M. D. Sutherland, *Tetrahedron Letters* 269 (1964)]. [e] See footnote *i* in Table 1.

methoxy product (ca. 75%) is the 3-methoxy derivative **2**. The main avenue of methylation for **1** is presented in the following scheme.



1 **2** **3** **4**

The methylation rate of the C-7 OH of 7-hydroxyjuglone (**5**) is so slow with diazomethane that the undesired addition of diazomethane to the unsubstituted quinone ring (one of the products is represented by structure **6**) takes precedence.[4]



5 **6**

Juglone derivatives

Demethylation. When the hydrolysis of 2- or 3-methoxyjuglone is monitored by TLC, it is apparent that the 3-MeO compound is 90% hydrolyzed in 2 min, while reaction of the 2-MeO isomer is only 20% complete at that time. Results are shown in Table 3. This large difference in hydrolysis rates is readily explained if protonation

TABLE 3. COMPARATIVE RATES OF HYDROLYSIS OF METHOXYJUGLONES IN ETHANOLIC HYDROCHLORIC ACID

Time (min)	Estimated hydrolysis (%) 2-Methoxyjuglone	3-Methoxyjuglone
2	20	90
8·5	75	99
20	99	100

[4] H. Brockmann, K. van der Merwe and A. Zeeck, *Chem. Ber.* **97**, 2555 (1964).

TABLE 4. HYDROLYSIS OF DI- AND TRIMETHOXYJUGLONES WITH ETHANOLIC HYDROCHLORIC ACID FOR FIFTEEN MINUTES

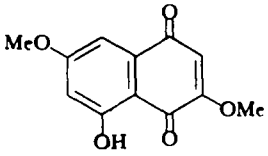
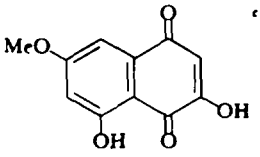
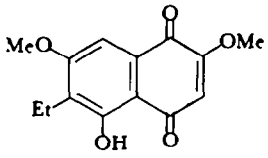
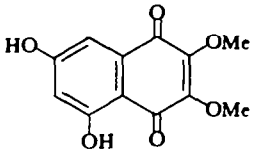
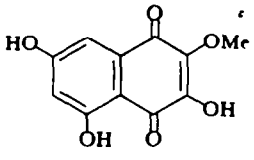
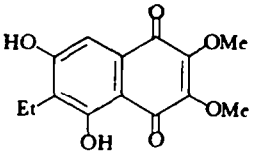
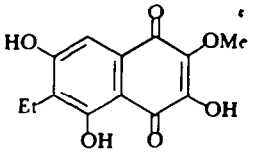
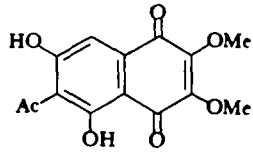
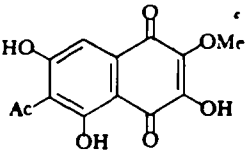
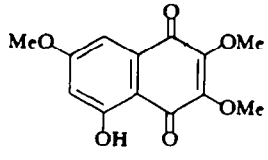
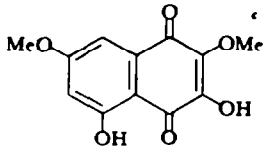
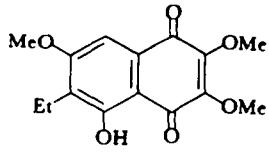
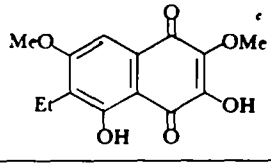
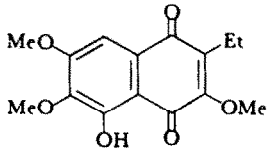
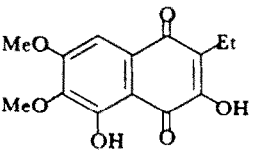
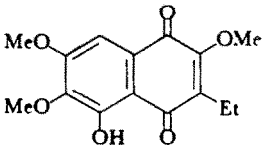
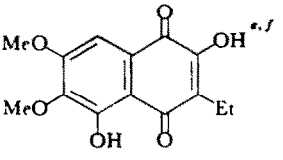
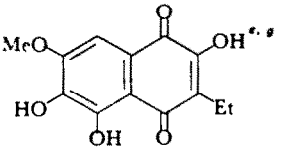
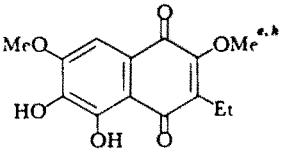
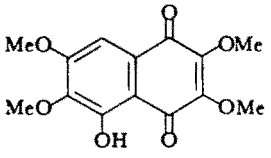
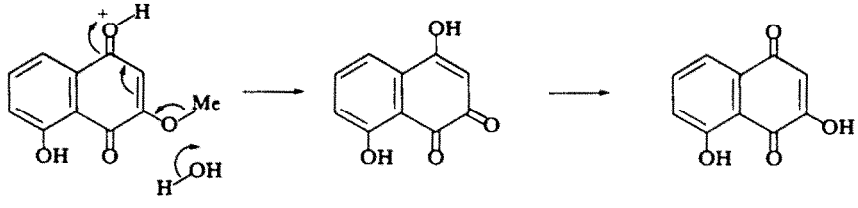
Reactant			Product		
Structure	Relative R_f[a]	M.p.	Structure	Relative R_f[a]	M.p.
	0·325	248–250°[b]		0·305	subl 235–240°
	0·510	189–189·5	mixture[d]		
	0·037	204–205°		0·016	240–243°
	0·065	207–209°		0·034	256–258°
	0·462	134–135°		0·243	238–240° (dec)
	0·381	113–114°		0·248	196–197°
	0·462	113–114°		0·344	183–184°

TABLE 4—*cont.*

Reactant			Product		
Structure	Relative R_f[a]	M.p.	Structure	Relative R_f[a]	M.p.
	0·480	141–142°		0·385	185–187°
	0·560	105°		0·429	165·5°
				0·218	232–233°
				0·278	160°
		140°	mixture[i]		

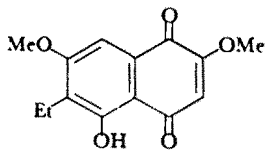
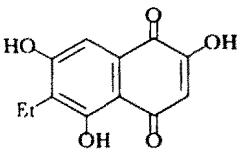
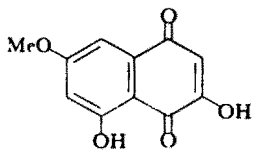
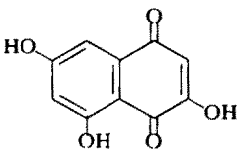
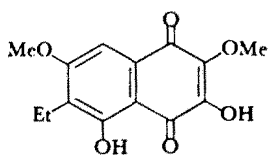
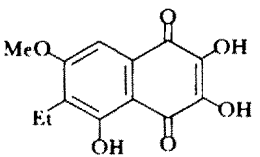
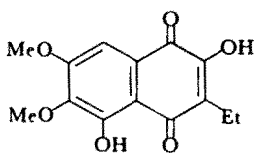
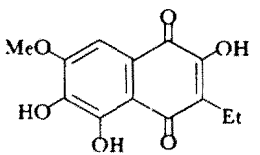
[a] Footnote *a* in Table 1. [b] Ref. 12. [c] Hydrolysis is generally complete even after 10 min and the yield is >95%. [d] After 20 min hydrolysis of the 2-MeO is <50%. [e] After 20 min 25% of the starting material remains unhydrolyzed. [f] 45% yield. [g] 15% yield. [h] 15% yield. [i] Insufficient material for structural analysis.

of the conjugated quinone carbonyl is postulated as a necessary step. A plausible mechanism is shown for 3-methoxyjuglone (7). Since protonation of the C-4 CO is



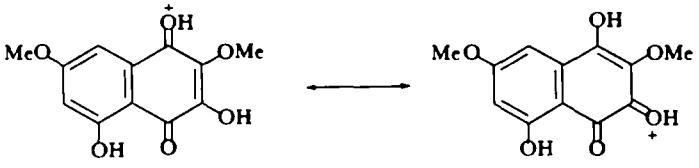
7

TABLE 5. HYDROLYSIS OF MONO- AND DIMETHOXYJUGLONES WITH ETHANOLIC HYDROCHLORIC ACID USING LONG REACTION PERIOD

Reactant			Product				
Structure	Relative R_f[a]	M.p.	Structure	Reaction period (hr)	Yield	Relative R_f[a]	M.p.
	0·510	189–189·5°		1·5	95%[b,c]	0·048	237° (dec)
	0·305	subl 235–240°		4	55%[b]		subl 235–255°
	0·344	183–184°		4	20%[b]		226 230° (dec)
	0·429	165·5°		4	98%[b]	0·218	232–233°

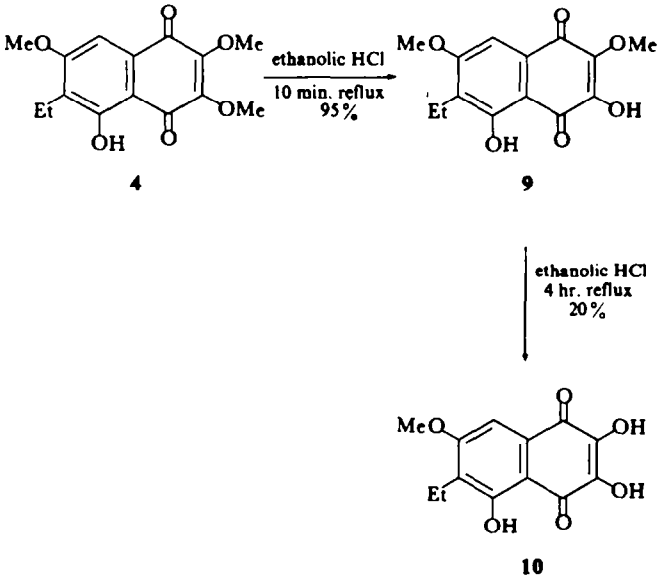
[a] Footnote *a* in Table 1. [b] The remainder is starting material. [c] The hydrolysis was conducted in a N atmosphere. In the presence of air 2,7-dihydroxy-3-ethylnaphthazarin was formed in ca. 15% yield.

necessary for hydrolysis of the 2-MeO compound, this may be impeded by hydrogen bonding of the C-4 CO to the C-5 OH. This situation remains unchanged in the hydrolysis of 2,3-dimethoxyjuglones. Only the 3-MeO group is cleanly hydrolyzed (ca. 95%) after a short hydrolysis period. The results are shown in Table 4. Further demethylation is retarded tremendously with ethanolic hydrochloric acid (Table 5) because of readier protonation of the non-hydrogen-bonded C-1 CO coupled with resonance stabilization of the protonated species (see for example **8a** ↔ **8b**). Further



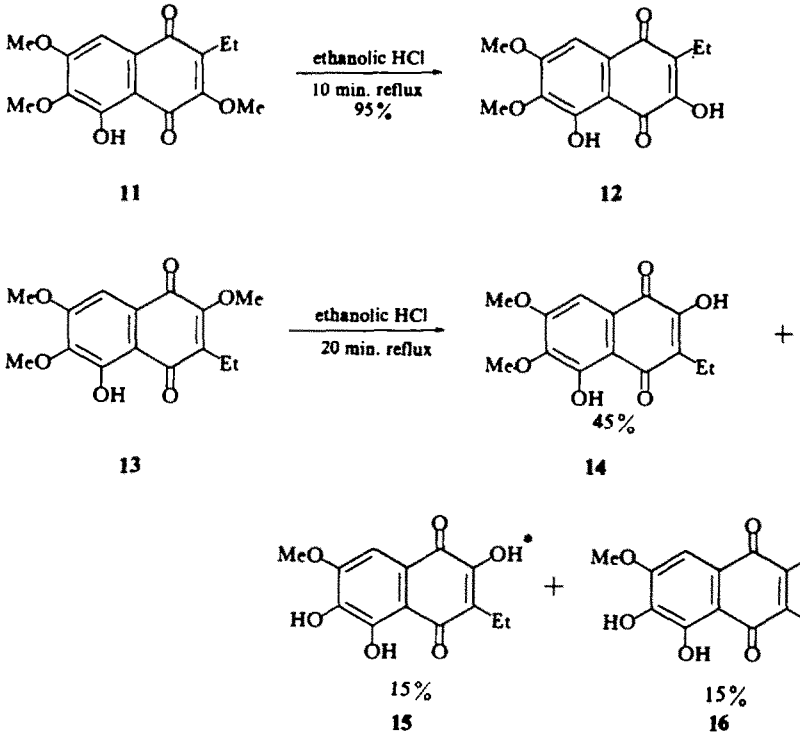
8a **8b**

demethylation of 2,7-*dimethoxy*-3-*hydroxy*-6-*ethyljuglone* (**9**) takes place to an extent of only 20% after 4 hr, the sole product being 2,3-*dihydroxy*-6-*ethyl*-7-*methoxyjuglone* (**10**). The demethylation pathway for **4** is presented in the following scheme.

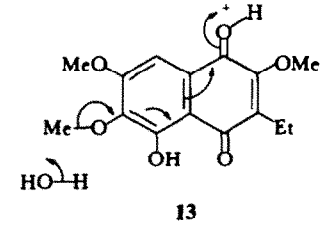


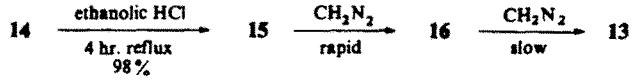
4 **9** **10**

Absence of a C-3 MeO leads to mixtures. Whereas the hydrolysis of 3,6,7-*trimethoxy*-2-*ethyljuglone* (**11**) to the expected 3-*hydroxy*-6,7-*dimethoxy*-2-*ethyljuglone* (**12**) is complete in 10 min, demethylation of the isomer 2,6,7-*trimethoxy*-3-*ethyljuglone* (**13**) is incomplete even after 20 min and affords approximately a 3:1:1 mixture of the corresponding 2-*hydroxy*- (**14**) 2,6-*dihydroxy*- (**15**) and 6-*hydroxy* (**16**) compounds. Most interesting is the selective demethylation of one of the aromatic



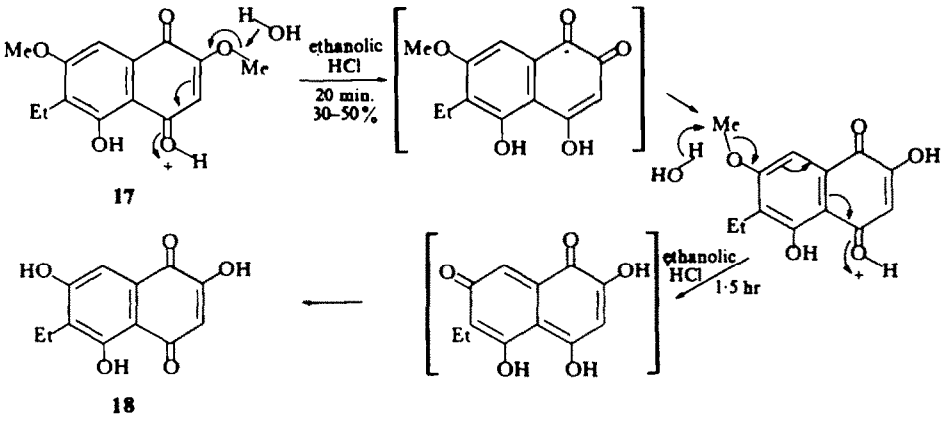
methoxyls which provides an illuminating corollary to the proposed mechanism for demethylation of quinoid methoxyls. The demethylation of the C-6 OMe can be envisioned by ready protonation of the C-1 CO followed by a nucleophilic attack of water on the C-6 OMe. Compound **15** is most likely formed by further demethylation of **16** rather than from the relatively slow hydrolysis of **14**.





When the C-6 OMe is absent, then demethylation of the C-7 OMe is possible. The complete demethylation of 2,7-dimethoxy-6-ethyljuglone (**17**) to 2,7-dihydroxy-6-ethyljuglone (**18**) is illustrative.

* Tentative structures with regard to placement of aromatic methoxyl.



Naphthazarin derivatives

Methylation with diazomethane. In contrast to the juglones where the acidities of the β-hydroxyls are markedly different depending on their location in the quinoid or benzenoid portion of the molecule, the β-hydroxyls of naphthazarins (5,8-dihydroxynaphthoquinones) have comparable acidities and methylation rates, since the rapid tautomerism of the naphthazarin system establishes benzenoid and quinoid character in both rings. The methylation rate appears to be comparable to that of an OH on the quinone portion of juglone. With diazomethane as the reagent the strongly hydrogen-bonded peri hydroxyls are not affected.

Naphthopurpurin (2-hydroxynaphthazarin) is rapidly converted to 2-methoxynaphthazarin. An excess of diazomethane must be avoided to prevent an addition reaction of diazomethane to the unsubstituted ring. The tautomeric nature of 2-methoxynaphthazarin[5] does impart enough quinoidal character to the unsubstituted ring to permit the addition reaction.

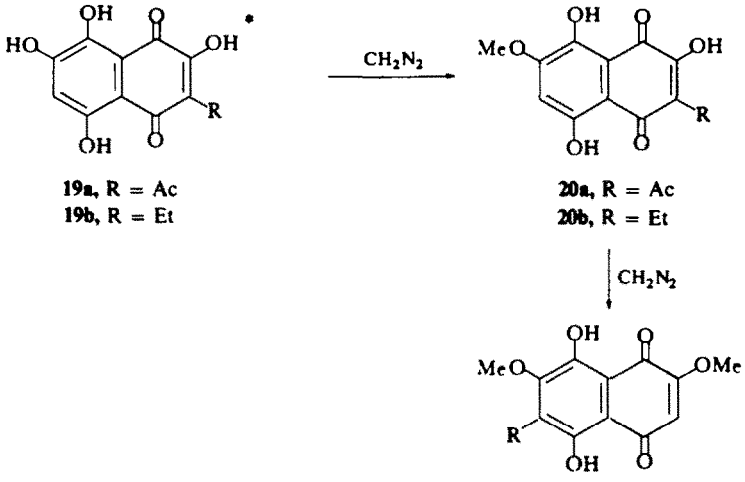
Methylation of 2,7- or 2,6-dihydroxynaphthazarin to the corresponding dimethyl derivative proceeds normally. Both hydroxyls appear to be methylated at virtually identical rates (Table 6). After monomethylation of 2,3-dihydroxynaphthazarin, however, methylation of the second OH is somewhat impeded by the steric crowding of the adjacent MeO (Table 6). The rate is not decreased sufficiently to allow appreciable diazomethane addition to the unsubstituted side.

2-Hydroxy-3-acetylnaphthazarin can be methylated quantitatively to 2-methoxy-3-acetylnaphthazarin[6] without diazomethane addition to the unsubstituted side even though the rate of methylation is decreased by the steric hindrance of the adjacent acetyl group. The 2-OH is not strongly hydrogen-bonded to the acetyl CO in ether–methanol.[5]

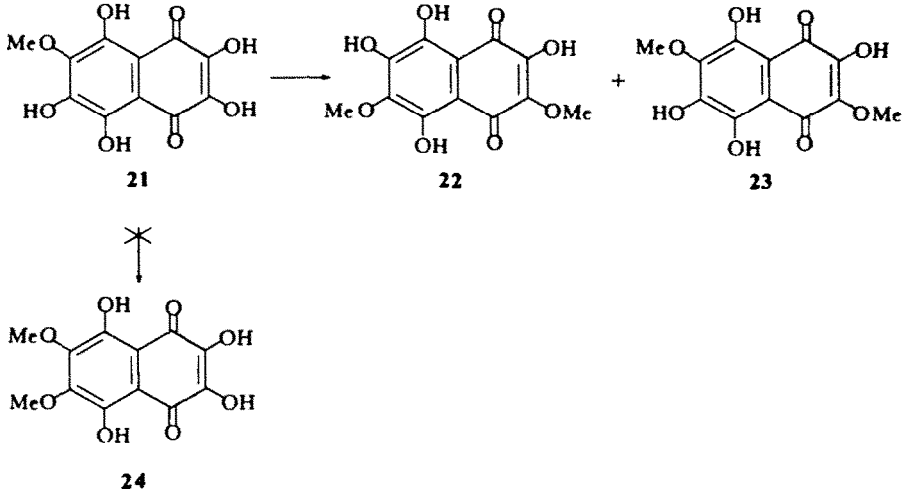
Even though the C-2 hydroxyls of 2,7-dihydroxy-3-acetylnaphthazarin (**19a**) and 2,7-dihydroxy-3-ethylnaphthazarin (**19b**) are more acidic due to their strong quinoidal nature, the C-7 hydroxyls of **19a** and **19b** are more readily attacked and the corresponding 7-MeO compounds can be isolated in good yield. Initial methylation of the C-2 OH of **19a** or **19b** is prevented due to the steric hindrance the adjacent Ac or

[5] R. E. Moore and P. J. Scheuer, *J. Org. Chem.* **31**, 3272 (1966).
[6] R. E. Moore, H. Singh, C. W. J. Chang and P. J. Scheuer, *J. Org. Chem.* **31**, 3638 (1966).

Et substituent. Both **20a** and **20b** form 2,7-dimethoxy derivatives on extended treatment with diazomethane.



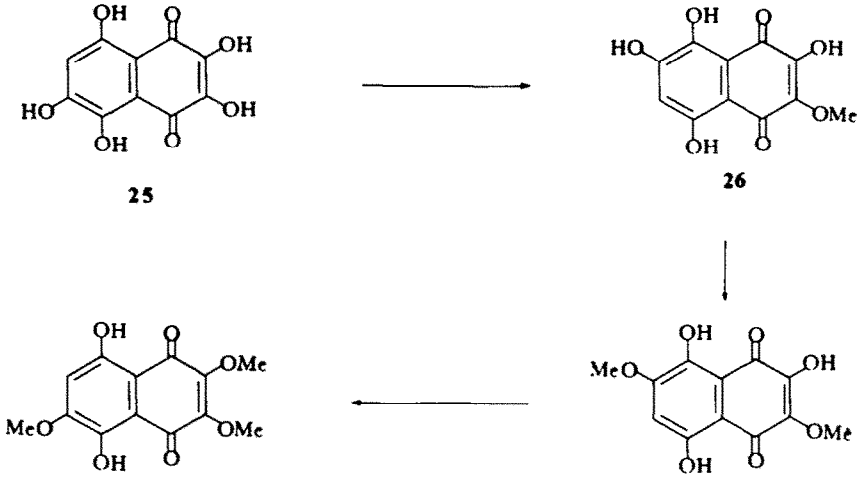
The C-2 OH of a 2,3,6-trihydroxynaphthazarin possessing either no substituent or a substituent such as Et or Ac at C-7 is clearly the most acidic one as only a single monomethoxy derivative can be isolated in all three cases. When, however, the C-7 substituent is OMe (**21**) then the C-2 or C-3 hydroxyls appear to have identical acidities and both the 3,6- (**22**) and 3,7-dimethoxy (**23**) compounds are obtained. Steric crowding of the C-6 OH in **21** by the adjacent MeO impedes the formation of the remaining dimethyl ether **24**.



After monomethylation of 2,3,6-trihydroxynaphthazarin (see **25** → **26**), further methylation is not entirely a function of acidity but rather relies on the steric and

* The principal tautomer is indicated in each naphthazarin formula throughout this paper. Substituents are numbered in order of decreasing attraction for quinoidal character: OH > Me > Ac > Et > H > Ac (Ref. 5).

hydrogen-bonded environment of the remaining hydroxyls. The methylation of the unhindered C-7 OH of **26** is preferred. The methylation of 2,7-dihydroxy-3-methoxy-6-ethylnaphthazarin (**28**), however, proceeds by two pathways as the bulkier Et



group (compared with the C-3 OMe) reduces methylation of the C-7 OH in **28** and the major dimethyl ether formed is **29**. The methylation pattern of **27** is shown in the following scheme.

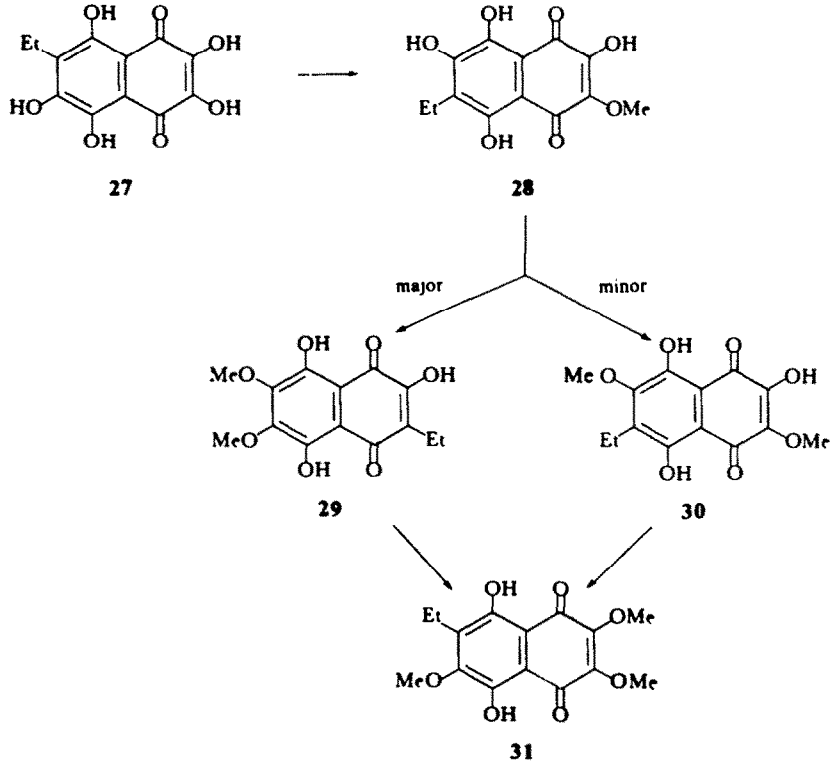
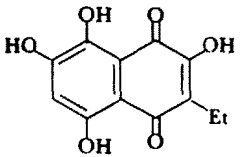
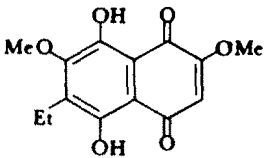
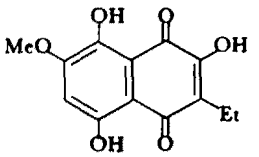
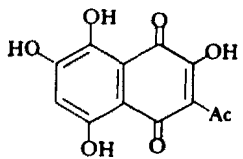
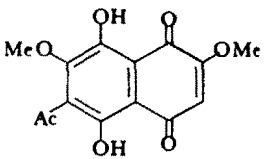
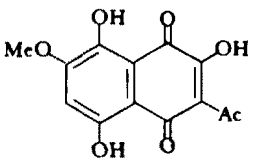


TABLE 6. PRODUCTS OF PARTIAL METHYLATION OF HYDROXYNAPHTHAZARINS WITH DIAZOMETHANE

Reactant			Products				
Structure	Relative R_f[a]	M.p.	Structure	Relative R_f[a]	M.p.	Approximate % composition[a]	Qualitative electronic spectrum $\lambda_{max}^{CHCl_3}$ mμ
	0·146	190–192°		0·463	145–147°[b]	40	548, 508, 479, 307
				0·445	230–232°	40	552, 508, 476, 312
	0·105	192–193°		0·290	181–182°	50	547, 502, 487, 306
				0·308	246–248°	40	

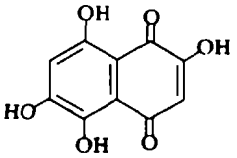
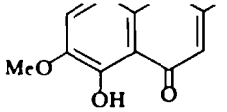
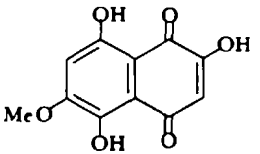
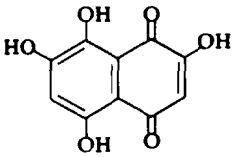
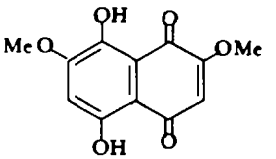
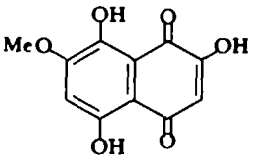
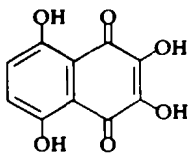
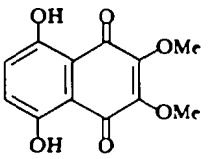
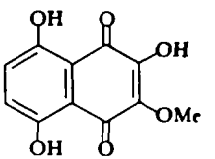
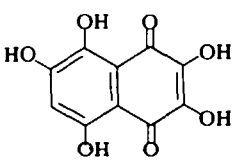
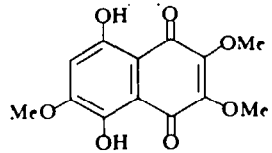
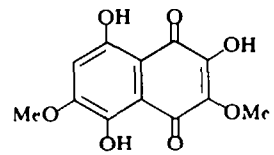
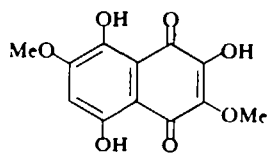
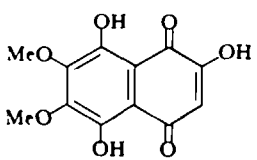
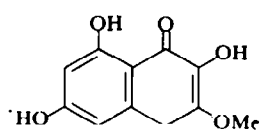
	0·059	270–285° (subl)		0·217	295–296°	50
				0·161	265–267°	20
	0·040	265–275° (subl)		0·257	273–275° with subl	50
				0·133	240–241°	20
	0·131	265°		0·577	136–137°	20
				0·308	181–182°	60

Table 6—*cont*

Reactant			Products				
Structure	Relative R_f[a]	M.p.	Structure	Relative R_f[a]	M.p.	Approximate % composition[e]	Qualitative electronic spectrum $\lambda_{max\ m\mu}^{Chf}$
	0·005	280–290° (subl)		0·228	161–162°	20	
				0·181	188–189°	15	
				0·167	193–194°	50	
				0·160	194–196°	5	
				not determined[f]	224°	trace	

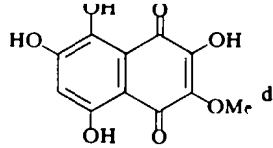
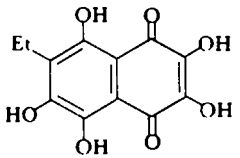
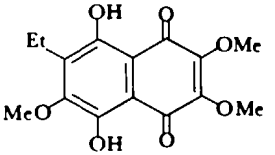
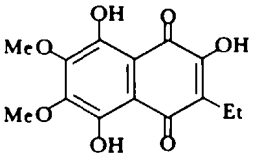
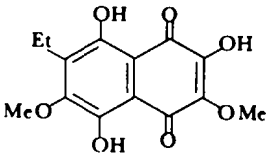
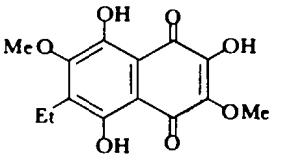
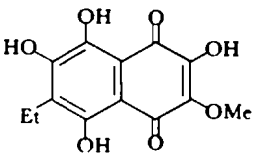
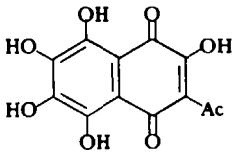
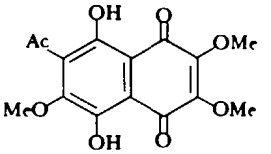
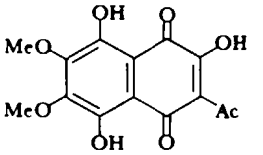
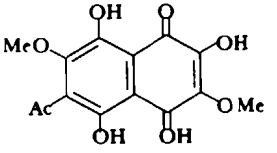
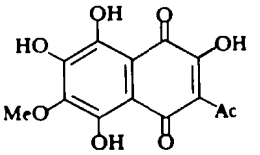
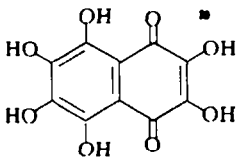
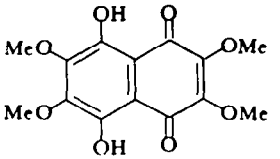
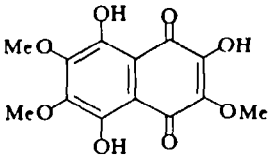
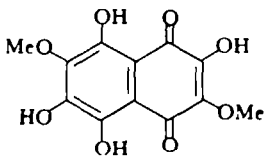
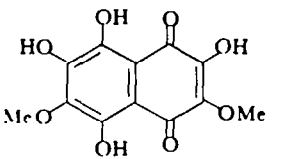
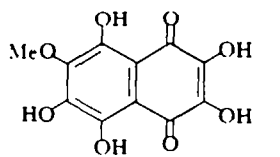
				not determined[f]	236–240°	trace	
	0·039	222–223°		0·493	131–132°	10	535, 501, 475, 320
				0·445	153–154°	30	527, 500, 468, 325
				0·388	116°	trace	527, 492, 460, 322
				0·297	152–154°	5	534(sh), 500, 470, 312
				0·105	187–188°	35	536(sh), 498, 470, 332

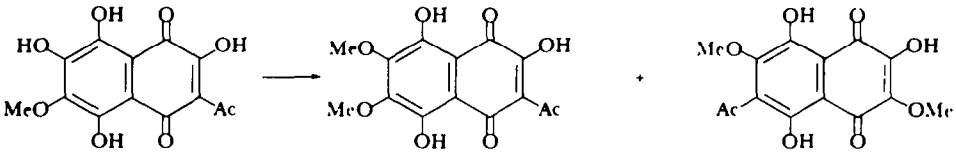
TABLE 6—*cont*

Reactant			Products				
Structure	Relative R_f[a]	M.p.	Structure	Relative R_f[a]	M.p.	Approximate % composition[c]	Qualitative electronic spectrum $\lambda^{CH}_{max\,m\mu}$
	0·038	246–248°		0·427[b]	126°	35	530(sh), 500, 470(sh), 320
				0·308[b]	224–227°	15	572(sh), 524(sh), 509, 312
				0·125	168–172° (dec)	15	530, 498, 462, 320
				0·098	190–193°	25	550, 510, 455, 328

	0·000	*subl* 300–320°		0·265	185–186°	20	526, 494, 466, 322
				0·110	134–135°	30	522, 490, 460, 333
				0·030[c]	252–254° (dec)	15	520, 482, 459, 338
				0·030[c]	218–219°	15	520(sh), 480, 463(sh), 342
				0·001	217–218°[d]	10	

[a] Footnote *a* in Table 1. [b] A mixture of these compounds does not separate readily with benzene; using a Chf system, 2-hydroxy-3-acetyl-6,7-dimethoxynaphthazarin now has a higher R_f than spinochrome *C* trimethyl ether. [c] Separated in Chf where the 3,7-dimethyl ether moves faster than than the 3,6-isomer. [d] Reported m.p. 218° [T. Mukai, *Bull. Chem. Soc. Japan* **33**, 453 (1960)]. [e] The remainder is starting material. [f] 2,6-Dihydroxy-3-methoxynaphthazarin has a larger R_f-value than 2,7-dihydroxy-3-methoxynaphthazarin in both benzene and Chf.

2,7-Dihydroxy-3-acetyl-6-methoxynaphthazarin (**32**) proceeds to the two dimethyl ethers **33** and **34** in equal amounts. Obviously the hydrogen-bonded nature of the C-2 OH of **32** is not important enough to permit only methylation of the C-7 OH.

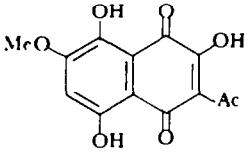
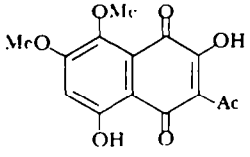
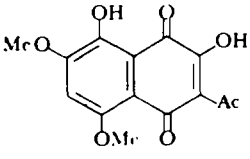
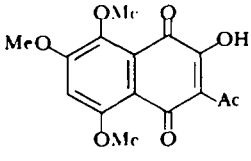


32 **33** **34**

Some physical properties and yields of partially methylated naphthazarin derivatives are given in Table 6.

Methylation with dimethyl sulfate. Unlike the diazomethane reaction which proceeds quantitatively with the juglone and naphthazarins, methylation with dimethyl sulfate is a low yield reaction. We carried it out only with spinochrome A

TABLE 7. METHYLATION OF SPINOCHROME A WITH DIMETHYL SULFATE.

Fraction	Products		Relative R_f [a]	Yield, %
1		**20a**	0·390	6
2		**35**		22
3	starting material	**19a**	0·105	8
4		**36**		1
5		**37**		5

[a] See footnote *a* in Table 1.

(2,7-dihydroxy-3-acetylnaphthazarin), where it leads to four products in addition to recovered starting material. As one might expect, the alkaline reaction conditions no longer protect the hydrogen-bonded peri hydroxyls from methylation as seen in the formation of a trimethoxy and two dimethoxy derivatives. It is not immediately apparent why no product with a 2-OMe group was isolated. The results are shown in Table 7.

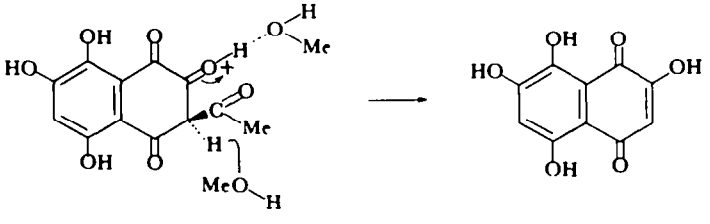
Methylation with methanol and hydrogen chloride. This reaction was explored only with spinochrome A, where in addition to methylation it leads to the loss of the Ac group.[7] The product composition (Table 8) provides a clue to the mechanism of

TABLE 8. PRODUCT COMPOSITION FOLLOWING REACTION OF SPINOCHROME A WITH METHANOL AND HYDROGEN CHLORIDE

Fraction	Products		Relative R_f*	Yield, %
1	7-Methoxy-2-hydroxy-3-acetylnaphthazarin	(**20a**)	0·390	15
2	2,7-Dimethoxynaphthazarin		0·257	6
3	2-Hydroxy-7-methoxynaphthazarin		0·133	3
4	Recovered spinochrome A	(**19a**)	0·105	57
5	2,7-Dihydroxynaphthazarin		0·040	3

* See footnote *a* in Table 1.

the reaction. Apparently deacetylation and methylation of the non-bonded 7-OH group take place at about the same rates. Accumulation of water in the reaction mixture prevents further reaction. However, when the reaction mixture is evaporated to dryness, the residue dissolved in dry methanol, and again saturated with dry hydrogen chloride, further reaction takes place and after repeated methylations



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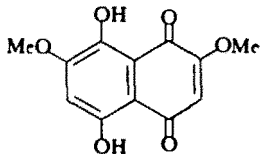
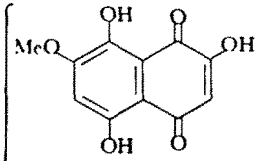
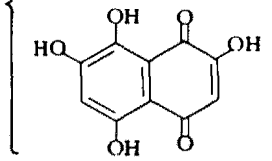
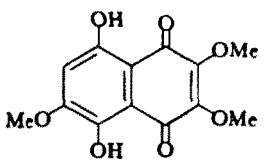
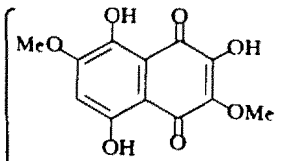
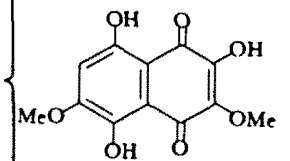
2,7-dimethoxynaphthazarin becomes the sole product. The loss of Ac may be pictured as proceeding *via* protonation at C-3 and a nucleophilic attack of methanol on the Ac function with elimination of methyl acetate and regeneration of the quinoid system. Esterification of the two β-hydroxyls results in the formation of 2,7-dimethoxynaphthazarin.

[7] C. W. J. Chang, R. E. Moore and P. J. Scheuer, *J. Am. Chem. Soc.* **86**, 2959 (1964).

TABLE 9. DEMETHYLATION OF DI-, TRI-, AND TETRAMETHOXYNAPHTHAZARINS

Reactant			Products							
Structure	Relative R_f	M.p.	Structure	Approx. % comp after minutes 20	35	45	60	90	Relative R_f [a]	M.p.
	0·257	273–275° with subl		35		50			0·133	240–241°
				5		40				subl 265–275°
	0·228	161–162°			1			0	0·167	193–194°
					10			0	0·181	188–189°

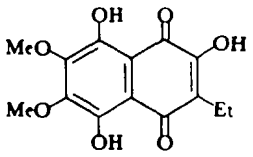
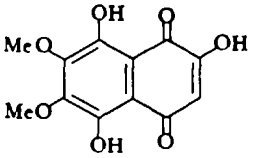
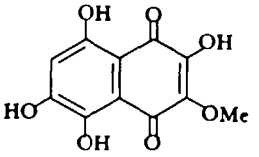
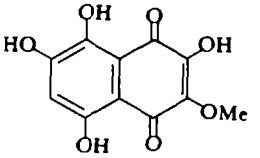
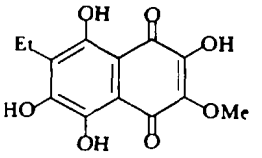
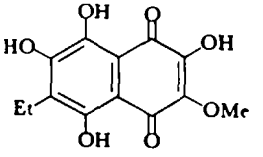
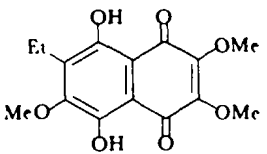
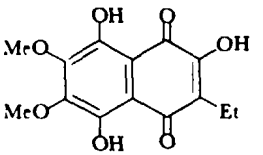
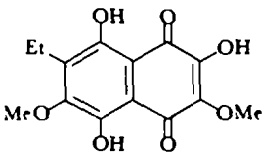
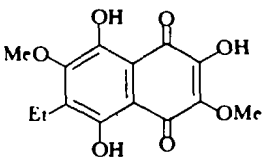
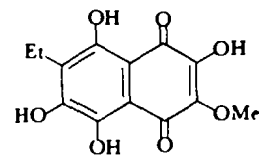
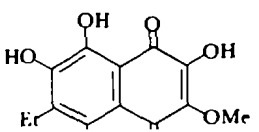
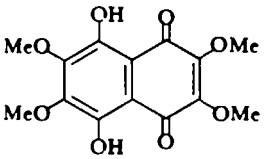
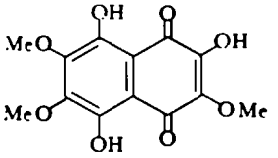
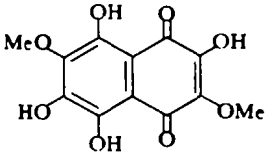
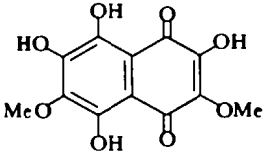
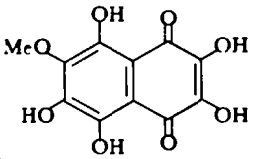
	0·445	153–154°			50		50	0·160	194–196°
					10		30		224°
					5		20		236–240°
				25		45		0·155	203–206°
				5		10		0·105	187–188°

Table 9—*cont.*

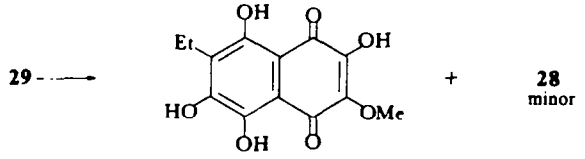
Reactant			Products							
Structure	Relative R_f [a]	M.p.	Structure	Approx. % comp after minutes 20	35	45	60	90	Relative R_f [a]	M.p.
	0·493	131–132°			20		20		0·445	153–254°
					15		15		0·388	116°
					10		15		0·297	152–154°
					0		25		0·155	203–206°
					0		10		0·105	187–188°

	0·265	185–186°		30	0·110	134–135°
				25	0·030	252–254° (dec)
				15	0·030	218–219°
				10	0·001	217–218°

[a] See footnote *a* in Table 1.

Methoxynaphthazarins

Demethylation. In the simplest case, that of 2-methoxynaphthazarin, formation of the demethylated product, naphthopurpurin, is complete after 15 min treatment with ethanolic hydrochloric acid. With 2,7-dimethoxynaphthazarin, however, (Table 9) demethylation is incomplete even after 45 min and although one would expect that the tautomerism of the naphthazarin system would impart quinoidal properties to both rings and thereby facilitate hydrolysis, complete reaction was achieved only after several hours' reflux. With two vicinal methoxyls, e.g. compound **29**, one OMe —the one leading to the 2,6-dihydroxy compound **38**—is lost in preference to the other which leads to the 2,7-dihydroxy derivative **28**. The reason for this preference is not immediately apparent. In the incomplete hydrolysis of **31**, compound **38** again is the predominant dihydroxy compound and its unquestioned precursor is **29**. In



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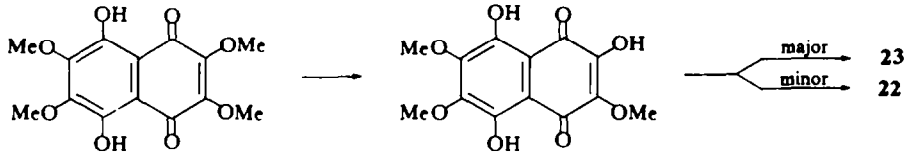
major

the case of 2,3,6,7-tetramethoxynaphthazarin (tetramethylspinochrome E) the formation of the symmetrical 2,6-dihydroxy isomer **23** again predominates.



Compound **20a** is the sole product when 2,7-dimethoxy-6-acetylnaphthazarin is treated with ethanolic hydrochloric acid. The hydrolysis proceeds with ease due to the stabilization of the product by tautomerism[5] and hydrogen-bonding.[8]

Peri methoxyls are very readily hydrolyzed to restore the stable naphthazarin system. Compound **20a** is the sole product from a mild hydrolysis of **35**. Similar treatment of the trimethyl derivative **37** yields **20a** as the major product.

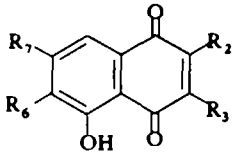
Structural determinations[9]

NMR spectra. Structures of the partially methylated polyhydroxyjuglones and naphthazarins were readily deduced from NMR data (Tables 10, 11). The chemical shifts of the various protons were essentially the same as those observed in appropriate model compounds.[5] The chemical shifts of the OMe signals of partially methylated polyhydroxynaphthazarins revealed which hydroxyls had been methylated and the observed values correlated well with calculated values (Table 12).[5]

[8] 2-Methoxyl-3-acetylnaphthazarin hydrolyzes to the corresponding 2-OH compound merely on standing in $CHCl_3$ soln for a few days.

[9] The structural determinations of most compounds reported here have been presented elsewhere. Structural elucidations of new compounds rely on previously published data and are presented here in abbreviated fashion.

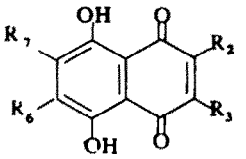
TABLE 10. NMR SPECTRA OF JUGLONES[a] OF STRUCTURE



Substituents				Chemical shift of R_i[b]					Chemical shift of	
R_2	R_3	R_6	R_7	H	OH	OMe	CH_2CH_3	CH_2CH_3	C-5 OH	C-8H
H	OH	H	OMe	6·27 (2) 6·60 (6)[c]	s.n.o.[d]	3·91			11·28	7·08[c]
OMe	Et	H	OH[e]	—	—	4·10	—	—	—	—
OH	H	Et	OMe[e]	6·21 (3)	—	3·98	—	1·12[f]	12·57	—
OMe	H	Et	OH[e]	—	—	3·90	—	—	—	—
OMe	OH	H	OMe	6·58	6·90[g]	4·15 (2) 3·90 (7)			11·31	7·23
OMe	OH	Et	OH			4·14				
OH	OMe	Et	OH			4·17				
OH	OH	Et	OMe			3·97	—	—	—	—
OMe	OH	Et	OMe		s.n.o.	4·14 (2) 3·96 (7)	2·67[h]	1·11[f]	11·38	7·24
OH	Et	OMe	OMe		s.n.o.	4·01 (6 or 7) 3·97 (6 or 7)	2·59[h]	1·13[f]	12·68	7·33
Et	OH	OMe	OMe		s.n.o.	4·01 (6 or 7) 3·97 (6 or 7)	2·57[h]	1·11[f]	11·18	7·32
OMe	Et	OMe	OMe[e]			4·12 (2) 4·00 (6,7)	2·58[h]	1·12[f]	12·48	7·23
Et	OMe	OMe	OMe[e]			4·11 (3) 4·01 (6 or 7) 3·99 (6 or 7)	—	1·13[f]	—	—
OMe	Et	OH	OMe		—	4·13 (2) 4·01 (7)	—	—	—	—
OH	Et	OH	OMe[e]		—	4·01	—	1·12[f]	—	—
OMe	OMe	OMe	OMe			4·08 (2,3) 3·98 (6 or 7) 3·97 (6 or 7)			—	—

[a] Only heretofore unreported spectral data are presented in this Table. NMR spectral data of all other juglones mentioned in this manuscript have been reported elsewhere by us. [b] Number in parentheses refers to position of substituent R_i. [c] Doublet, J = 2·5 c/s. [d] Signal not observed. [e] Insufficient material or solubility difficulties prevented observance of the entire spectrum. [f] Triplet, J = 7·5 c/s. [g] Broad band. [h] Quartet, J = 7·5 c/s.

TABLE 11. NMR SPECTRA OF NAPHTHAZARINS[a] OF STRUCTURE



Substituents			Chemical shift of R_1						Chemical shift of peri hydroxyl	
R_5	R_6	R_7	H	OH	OMe	Ac	CH_2CH_3	CH_2CH_3	C-5	C-8
Et	H	OMe	6·58	s.n.o.[b]	3·96		2·63[c]	1·14[d]	13·33	
Et	OMe	OMe		s.n.o.	4·13 (6)[e]		2·60[c]	1·13[d]	13·48	12·15
					4·04 (7)					
OMe	OMe	Et			4·17 (3)					
					4·08 (6)					
OMe	Et	OMe			4·20 (3)		2·75[c]	1·16[d]		
					4·04 (7)					
OMe	Et	OH			4·20		2·68[c]	1·16[d]		
OMe	OH	Et			4·13					
Ac	OMe	OMe		14·28	4·18 (6)	2·83			12·75	12·75
									or	or
					4·07 (7)				12·90	12·90
OMe	Ac	OMe		a.n.o.	4.20 (3)	2·51			12·68	12·00
					4·04 (7)					
Ac	OMe	OH[e]			4·24	2·83				
H	OMe	OMe	6·45	s.n.o.	4·15 (6)					
					4·04 (7)					
OMe	OH	H[e]	6·60		4·13					
OMe	H	OH[e]			4·25					

[a] Only hitherto unreported spectra are presented in this Table. NMR spectra of all other naphthazarins mentioned in this paper have been reported elsewhere by us. [b] Signal not observed. [c] Quartet, J = 7·5 c/s. [d] Triplet, J = 7·5 c/s. [e] Insufficient material or solubility difficulties prevented observance of the entire spectrum.

TABLE 12. COMPARISON OF OBSERVED AND CALCULATED CHEMICAL SHIFTS FOR METHOXY PROTONS OF SOME TRI- AND TETRASUBSTITUTED NAPHTHAZARINS

Compound		Methoxyl chemical shift in $CDCl_3$ Calc[a]	Observed	Position of OMe
2-Hydroxy-3-ethyl-6,7-dimethoxynaphthazarin	(29)	4·12 − 0·03 + 0·04 = 4·13 4·12 − 0·08 = 4·04	4·13 4·04	6 7
2-Hydroxy-3,6-dimethoxy-7-ethylnaphthazarin		4·22 − 0·06 = 4·16 4·13 − 0·06 + 0·04 = 4·11	4·17 4·08	3 6
2-Hydroxy-3,7-dimethoxy-6-ethylnaphthazarin	(30)	4·22 − 0·03 + 0·01 = 4·20 4·13 − 0·08 = 4·05	4·20 4·04	3 7
2,7-Dihydroxy-3-methoxy 6-ethylnaphthazarin	(28)	4·22 − 0·03 + 0·04 = 4·23	4·20	3
2,6-Dihydroxy-3-methoxy-7-ethylnaphthazarin		4·22 − 0·08 = 4·14	4·13	3
2-Hydroxy-3-acetyl-6,7-dimethoxynaphthazarin	(33)	4·12 + 0·04 = 4·16 4·12 − 0·08 − 0·01 = 4·03	4·18 4·07	6 7
2-Hydroxy-3,7-dimethoxy-6-acetylnaphthazarin	(34)	4·22 + 0·00 = 4·22 4·13 − 0·08 = 4·05	4·20 4·04	3 7
2,7-Dihydroxy-3-acetyl-6-methoxynaphthazarin	(32)	4·22 + 0·04 = 4·26	4·24	6
2-Hydroxy-6,7-dimethoxy-naphthazarin		4·12 + 0·04 = 4·16 4·12 − 0·08 = 4·04	4·15 4·04	6 7
2,6-Dihydroxy-3-methoxy-naphthazarin		4·22 − 0·08 = 4·14	4·13	3
2,7-Dihydroxy-3-methoxy-naphthazarin	(26)	4·22 + 0·04 = 4·26	4·25	3
2,7-Dihydroxy-3,6-dimethoxy-naphthazarin		4·22 − 0·06 + 0·04 = 4·20	4·17	3,6
2,6-Dihydroxy-3,7-dimethoxy-naphthazarin		4·22 − 0·08 = 4·14	4·13	3,7
2,3-Dihydroxy-6,7-dimethoxy-naphthazarin		4·12 − 0·08 = 4·04	n.d.[b]	6,7
2,3,6-Trihydroxy-7-methoxy-naphthazarin		4·22 − 0·08 = 4·14	n.d.	7

[a] For explanation of calculation see Ref. 5. [b] Not determined.

The selective demethylation of the 3-position of a 2,3-dimethoxyjuglone was confirmed from the position of the peri-OH signal. The presence of a 3-OH caused a pronounced diamagnetic shift of the C-5 OH proton (normally around δ 12 or lower field) to the neighborhood of δ 11. The isomeric compounds 10 and 12 were differentiated by the respective 11·18 and 12·68 ppm signals for the C-5 OH protons. Similarly, the structure of **2** and 3,7-dihydroxy-2-methoxy-6-ethyljuglone could be distinguished from the position of the peri-hydroxyl signal (Table 10).

The peri OH of **36** does not resonate near δ 11, presumably due to the operation of a different tautomerism and anisotropy in the quinoidal system by the presence of the C-2 Ac. Note, however, that the C-5 OH of **36** resonates at a higher field (δ 13·03) than that of **35** (δ 13·50). The structures of the two spinochrome A dimethyl ethers **35** and **36** were further supported by examination of their NMR spectra in benzene

TABLE 13. CHEMICAL SHIFTS AND SOLVENT SHIFTS OF METHOXYL RESONANCES IN SOME DIMETHYL DERIVATIVES OF SPINOCHROME A

Compound	$\delta^{a}_{CDCl_3}$	$\delta^{a}_{C_6H_6}$	$\Delta^{CDCl_3,b}_{C_6H_6}$
35	3·96 (7)	3·03 (7)	+ 0·93
	3·88 (8)	3·78 (8)	+ 0·10[c]
36	3·98 (6 or 8)	3·16 (6)[d]	+ 0·82 and + 0·77
	4·00 (6 or 8)	3·21	or + 0·84 and + 0·79[e]
		3·30 (8)[f]	+ 0·68 and + 0·58
		3·40	or + 0·70 and + 0·60[e]

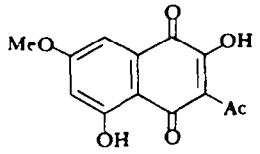
[a] Numbers in parentheses refer to the position of attachment of the methoxyls on the juglone system. [b] $\Delta^{CDCl_3}_{C_6H_6} = \delta_{CDCl_3} - \delta_{C_6H_6}$. [c] A + 0·11 shift for the C-1 OMe of 1,2-dimethoxyanthraquinone is reported in Ref. 10. [d] The relative intensity of the 3·16 ppm peak to the 3·21 ppm peak is 2 to 1. [e] Tentative assignments. [f] The relative intensity of the 3·30 ppm peak to the 3·40 ppm peak is 2 to 1.

and deuteriochloroform (Table 13).[10] Whereas most unhindered methoxyls attached to a naphthoquinone system exhibit a diamagnetic shift of ca. 1 ppm in benzene compared to deuteriochloroform, the hindered C-8 methoxyls of **35** and **37** show a relatively small shift in benzene. It is interesting to note that the OMe signals in the spectrum of **36** are doubled in benzene solvent but not in deuteriochloroform.

The chemical shift of the ring proton of compounds **35**, **36**, and **37** is consistently around 6·8 ppm showing benzenoid character in this ring. The hydrogen-bonded acetyl of **35** is denoted by the δ 2·83 signal, but the C-2 Ac protons of **36** show a signal at higher field (δ 2·70). Presumably a shielding effect is exerted on the Ac protons by the peri-OMe of **36**. A comparable Ac chemical shift is observed in the spectrum of **37**; actually this signal is doubled for reasons already described,[5] but the center of the doublet is at δ 2·72.

B. *Electronic spectra.* Most new compounds exhibited the expected electronic spectra.[11]

In the spectrum of compound **36** peri methylation has produced about a 30 mμ hypsochromic shift of the visible peak[12] compared with that of **20a**, but the remainder of the spectrum is virtually identical with that of **20a**. The electronic spectrum of **35** is quite different from that of **36**. The C-8 OMe of **35** is sterically hindered and the



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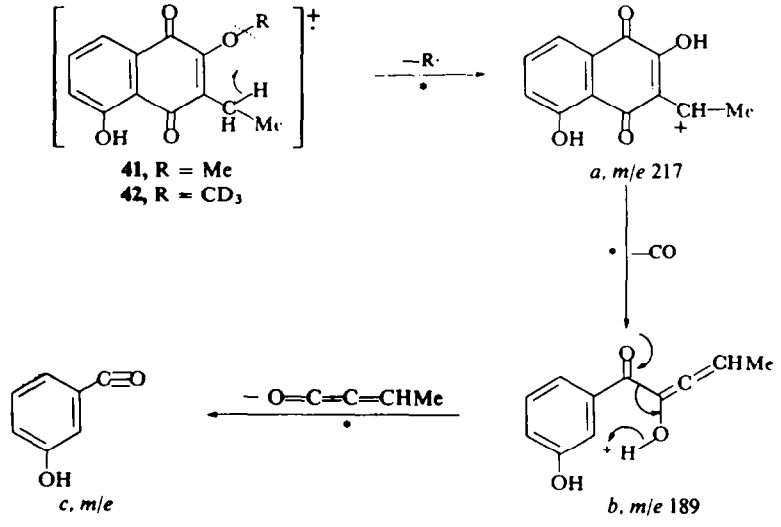
[10] J. H. Bowie, D. W. Cameron, P. E. Schütz, D. H. Williams and N. S. Bhacca, *Tetrahedron* **22**, 1771 (1966).
[11] I. Singh, R. E. Moore, C. W. J. Chang, R. T. Ogata, and P. J. Scheuer, in preparation.
[12] 5-Methoxy-1,4-naphthoquinone exhibits its visible band at 396 mμ, a 33 mμ hypsochromic shift as compared with juglone (429 mμ).

O-Me bond is forced from the plane of the ring. As a result the orbitals of the non-bonding p-electrons of the OMe oxygen suffer decreased overlap with the π-electrons of the conjugated ring system. If one assumes that this hindered C-8 OMe produces no effect on the electronic spectrum, the shape of the spectrum should be very similar to that of 2-hydroxy-3-acetyl-7-methoxyjuglone (**39**). This is indeed the case.[13] It should be pointed out that the C-8 OMe does exert some effect on the chromophore, but its only appreciable effect is on the position of the visible peak. As a result the visible peak in the spectrum of **35** is bathochromically displaced by about 20–25 mμ from that of **39**.[14, 15]

C. *Mass spectra:* The mol wts of all new compounds were confirmed by mass spectral analyses. Generally the molecular ions form the base peaks in the mass spectra. The fragmentations upon electron-impact for the most part follows the rules and generalizations enumerated by Williams *et al.*[16] and more recently by us.[17]

While the fragmentation of 2-hydroxy-3-ethyljuglone (**40**) is initiated by loss of a Me radical from the Et group,[6] the Me radical is not initially expelled from the Et



[a] Transitions supported by a metastable ion are marked by asterisks. In the absence of ^{18}O or ^{13}C labelling, the site of carbon monoxide expulsion from the molecule is depicted in an arbitrary manner for illustrative purposes only.

[13] Unpublished results from this Laboratory. 2,7-Dihydroxy-3-acetyljuglone has recently been isolated from the spines of *Echinothrix diadema* Linn. and the electronic spectrum of its methyl ether derivative **39** will be presented in a subsequent publication.

[14] Actually this is not an unreasonably large shift. The introduction of an acetoxyl group, a substituent which is known to contribute very little to the chromogen, in the C-5 position of 1,4-naphthoquinone causes a 10 mμ bathochromic shift of the 335 mμ peak and no appreciable effect on the remainder of the spectrum (see Ref. 11).

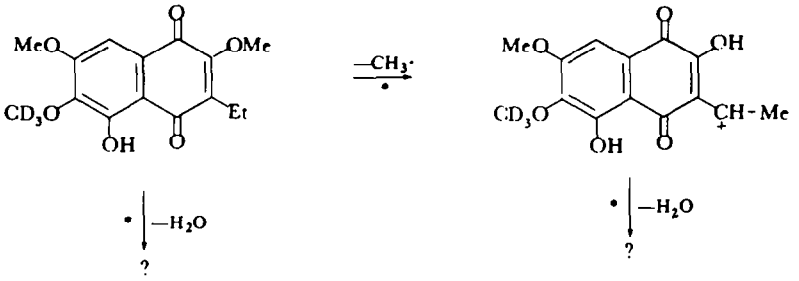
[15] For further examples of the effect of a hindered peri methoxyl on the electronic spectra, compare the spectra of diomelquinone A derivatives [G. S. Sidhu and A. V. B. Sankarum, *Ann.* **691**, 172 (1966)] with those of appropriate model compounds in Ref. 11.

[16] J. H. Bowie, D. H. Cameron and D. H. Williams, *J. Am. Chem. Soc.* **87**, 5094 (1965).

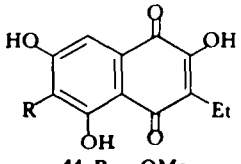
[17] D. Becher, C. Djerassi, R. E. Moore, H. Singh and P. J. Scheuer, *J. Org. Chem.* **31**, 3650 (1966).

group in the mass spectrum of 2-methoxy-3-ethyljuglone of (**41**). The mass spectrum of *2-methoxy-*d_3*-3-ethyljuglone* (**42**) clearly shows that the OMe group is involved. The benzyl ion *a* which is formed consequently loses carbon monoxide to form ion *b* followed by a hydrogen-rearrangement process analogous to that of **40** to form *c*. The principal pathway of disintegration for compounds **11**, **13**, and **16** is the same as for **40**.

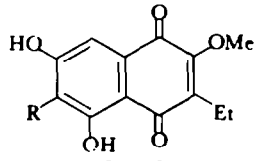
It was hoped that the structure of **16** could be secured by the mass spectrum of 2,7-dimethoxy-6-methoxy-d_3-3-ethyljuglone (**43**). Loss of HDO from the parent and M-15 ions would reflect the vicinity of the MeO-d_3 group and the peri-OH.[17] The actual loss of water (M-18) from the parent and M-15 ions could not be explained. Structures **15** and **16** must therefore be accepted for the present as tentative.



D. *R_f-Values.* The relative R_f-values for compounds **15** and **16** are 0·218 and 0·278 respectively. If the methoxyls were not at the 7-positions, but rather at the 6-positions, then one would expect the R_f-values of **44** and **45** to be slightly less than those of 2,7-dihydroxy-3-ethyljuglone (**46**), R_f 0·082, and 2-methoxy-3-ethyl-7-hydroxyjuglone (**47**), R_f 0·139, respectively. The smaller R_f-values would be due to the polarity of the methoxyls at the 6-position. This is definitely not the case as the R_f-values of **15** and **16** are much greater than those for **44** and **45**. The OMe group may be at the 7-position to reduce the polarity of compounds **15** and **16**.



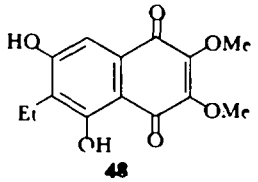
44, R = OMe
46, R = H



45, R = OMe
47, R = H

E. *Sodium borohydride reduction.* The structures of a few selected partially methylated naphthazarins are rigorously secured from chemical evidence. Compound **32**, for example, is readily reduced with sodium borohydride to **28**. Compound **30** gives 2,6-dimethoxy-7-ethylnaphthazarin[6] as one of the reduction products proving conclusively the positions of the two methoxyls. Compound **29** is reduced with sodium borohydride to give 2,3-dimethoxy-6-ethyl-7-hydroxyjuglone (**48**), identical in all respects with the dimethyl ether of the natural pigment.[18]

[18] R. E. Moore, H. Singh and P. J. Scheuer, *J. Org. Chem.* **31**, 3645 (1966).



48

EXPERIMENTAL[19]

Methylation of hydroxyjuglones with diazomethane

The juglone was dissolved in MeOH–ether and treated with a small excess of diazomethane in ether. If only methylation of the OH groups on the quinone ring was desired, the soln was evaporated immediately *in vacuo* and the product was purified by column or TLC on acid-treated, deactivated silica gel using benzene or $CHCl_3$ as the eluant. Generally the product was formed in 95–100% yield. The results of several methylations of hydroxyjuglones are summarized in Table 1. To methylate the phenolic OH groups which are not strongly hydrogen-bonded, the soln of the juglone was allowed to stand for 15–30 min in contact with excess diazomethane and then evaporated *in vacuo*. The product was purified by column or TLC on acid-treated, deactivated silica gel using benzene. The yield of the product was usually > 90%. The results of some methylations are given in Table 2.

Acid hydrolysis of methoxyjuglones

A. Monomethoxyjuglones. A soln of 5 mg methoxyjuglone in 5 ml EtOH was brought to boiling and 5 ml of conc HCl was added dropwise over 1·5 min. When 2, 8·5, and 20 min had elapsed after the completion of the acid addition, aliquots were removed, diluted with water, and extracted with benzene. The benzene extract was examined by TLC on acid-treated, deactivated silica gel to determine the amount of hydrolysis to the corresponding hydroxyjuglone. The results of the hydrolyses of two monomethoxyjuglones to the corresponding monohydroxyjuglones are given in Table 3.

B. Dimethoxyjuglones and trimethoxyjuglones. The juglone (25 mg) was dissolved in boiling EtOH (15 ml) and an equal volume of conc HCl was added dropwise over 1·5 min. The soln was boiled in an open beaker for 15 min, diluted with water, and the compound was extracted into benzene–ether. The product was purified by column or TLC on silica gel using benzene or $CHCl_3$ as the eluant and usually >90% yield of a monodemethyl compound was obtained when the starting material possessed a MeO group in the 3-position. If the starting material did not contain a MeO group in the 3-position, a mixture of starting material and products was obtained. The results of several hydrolyses are given in Table 4.

Prolonged treatment with ethanolic HCl (1–4 hr) effected removal of another Me group in some cases. The results are presented in Table 5.

Partial methylation of hydroxynaphthazarins with diazomethane

The naphthazarin was dissolved in ether or ether–MeOH and treated slowly with diazomethane. The progress of the methylation was followed on a thin-layer plate and was terminated in most cases when the amount of starting material had become small. The solvent was removed *in vacuo* and the methylated products were separated by preparative TLC. In Table 6 are given the results of several methylation of hydroxynaphthazarins.

Methylation of spinochrome A (*2,7-dihydroxy-3-acetylnaphthazarin*) *with dimethyl sulfate*

To a stirred soln of 500 mg spinochrome A in 50 ml dry acetone was added 25 g anhyds K_2CO_3 and

[19] UV-visible spectra were determined in MeOH or $CHCl_3$ on a Cary 14 spectrophotometer; NMR spectra on a Varian A-60 instrument in $CDCl_3$ unless otherwise noted. Combustion analyses by Berkeley Analytical Laboratory, Berkeley, Cal. Generally, few were determined in order to conserve material and since these highly oxygenated compounds are known to combust poorly. Mass spectrometric analyses confirmed the mol wts of all new compounds. Mass spectra were determined at Stanford University on an A.E.I. MS-9 instrument and at Hawaii on a Hitachi Perkin-Elmer RMU-6D2-s spectrometer operating with an ionization energy of 70 eV. The temp of the ion source was about 200° for both instruments.

15 ml of freshly distilled Me_2SO_4 and the mixture was kept under an argon atmosphere in the dark for 24 hr. An additional 50 ml of acetone, 25 g K_2CO_3, and 15 ml Me_2SO_4 was added and the pasty slurry was refluxed for 3 hr. Water was added and the soln was extracted with ether. The basic phase was acidified with phosphoric acid and extracted with ether. The combined extracts were evaporated and the residual oil chromatographed on a 80 cm × 5 cm column of acid-treated, deactivated silica gel. 5 bands could be eluted with benzene (Table 7). Fraction 3 gave 40 mg of unreacted spinochrome A.

Fraction 1 crystallized from $CHCl_3$–isooctane to give 48 mg of *2-hydroxy-3-acetyl-7-methoxynaphthazarin* (**20a**) as dark brown needles, m.p. 246–248°. UV spectrum in MeOH: λ_{max} 508 mμ (ε 4900), 313 (12,000), 271 (14,800); λ_{min} 371 mμ (ε 1730), 288 (10,500). UV spectrum in $CHCl_3$: λ_{max} 525, 318 mμ (relative intensities 1·00, 2·45); λ_{min} 410, 284 mμ (relative intensities 0·37, 1·91). (Found: C, 56·22; H, 3·85. $C_{13}H_{10}O_7$ requires: C, 56·12; H, 3·62%).[20]

Fraction 2 crystallized from $CHCl_3$–isooctane to give 109 mg of *2-hydroxy-3-acetyl-7,8-dimethoxyjuglone* (**35**) as long red needles, m.p. 224–227°. NMR spectrum in $CDCl_3$: C-2 OH, δ 17·20; C-3 Ac, 2·83; C-5 OH, 13·50; C-6 H, 6·75; C-7 OMe, 3·96; C-8 OMe, 3·88. UV spectrum in MeOH: λ_{max} 450 mμ (ε 2700), 302 (15,000), 263 (12,000); λ_{min} 408 mμ (ε 2400), 279 (10,500). UV spectrum in $CHCl_3$: λ_{max} 450, 307, 274 mμ (relative intensities 3·2, 14·2, 13·8); λ_{min} 380, 283, 253 mμ (relative intensities 1·9, 12·6, 11·3). (Found: C, 57·59; H, 4·55. $C_{14}H_{12}O_7$ requires: C, 57·54; H, 4·14%).[20]

Fraction 4 crystallized from $CHCl_3$ to give 5 mg of *2-acetyl-3-hydroxy-6,8-dimethoxyjuglone* (**36**) as red needles, m.p. 216–217°. NMR spectrum in acetone-d_6: C-2 Ac, δ 2·70; C-7 H, 7·21; C-6 OMe, 4·05 or 4·08; C-8 OMe, 4·05 or 4·08. NMR spectrum in $CDCL_3$: C-2 Ac, δ 2·70; C-6 OMe, 3·98 or 4·00; C-8 OMe, 3·98 or 4·00; C-7 H, 6·83; C-5 OH, 13·03. UV spectrum in $CHCl_3$: λ_{max} 490, 320, 270 mμ (relative intensities 4·5, 12·2, 11·1); λ_{min} 395, 290, 252 mμ (relative intensities 1·4, 10·3, 8·4).[20]

Fraction 5 crystallized from $CHCl_3$–isooctane to give 25 mg of *2-hydroxy-3-acetyl-5,7,8-trimethoxy-1,4-naphthoquinone* (**37**) as dark orange needles, m.p. 255–257°. NMR spectrum in $CDCl_3$: C-3 Ac, δ 2·68 and 2·76 (relative intensities 1:1); C-8 OMe, 3·88; C-5 and C-7 OMe's, 3·99; C-6 H, 6·78 and 6·83 (relative intensities 1:1). UV spectrum in MeOH: λ_{max} 435, 371, 303 mμ; λ_{min} 405, 350 mμ. (Found: C, 58·67; H, 4·68. $C_{15}H_{14}O_7$ requires: C, 58·82; H, 4·61%).[20]

Reaction of spinochrome A (*2,7-dihydroxy-3-acetylnaphthazarin*) *with methanolic hydrogen chloride*

A soln of 100 mg spinochrome A in 40 ml abs MeOH was saturated with dry HCl. The reaction vessel was well stoppered and allowed to stand in a water bath at 50–60° for 24 hr. At the end of this time thin-layer plates showed a 5 component mixture and further standing of the reaction mixture did not alter the composition. If the reaction mixture was evaporated to dryness and the residue chromatographed on deactivated acid-washed silica gel, the five components (Table 8) could be readily separated using benzene as the eluent: (1) 15 mg of **20a** (purple band), m.p. 246–248° needles from $CHCl_3$–hexane; (2) 6 mg 2,7-dimethoxynaphthazarin (orange–red band); (3) 3 mg 2-hydroxy-7-methoxynaphthazarin (red band), red-brown needles from $CHCl_3$, m.p. 235–236°; (4) 57 mg of recovered spinochrome A (purple band); and (5) 3 mg 2,7-dihydroxynaphthazarin (red band), needles from $CHCl_3$, m.p. 269° (dec).

The reaction, however, could be carried out in favor of 2,7-dimethoxynaphthazarin formation by evaporating the reaction mixture to dryness after 10 hr standing, redissolving the residue in MeOH and saturating the soln with dry HCl. After 10 hr standing the soln was again evaporated to dryness and the process repeated several times until TLC showed complete elimination of purple spots.

Acid hydrolysis of methoxynaphthazarin

A *Methoxynaphthazarin.* A soln of 25 mg 2-methoxynaphthazarin in 15 ml EtOH was brought to boiling and 15 ml conc HCl was added over 1–2 min. Boiling was continued for 15 min after which time hydrolysis to naphthopurpurin was complete as indicated by TLC.

B. *Di-, tri-, and tetramethoxynaphthazarins.* The methoxynaphthazarin was treated as described above and hydrolysis was conducted from 20–90 min. The mixture of products were separated by preparative TLC using benzene, $CHCl_3$, or CCl_4. The results of several demethylations are given in Table 9.

Acid hydrolysis of 2-hydroxy-3-acetyl-7,8-dimethoxyjuglone (**35**)

2-Hydroxy-3-acetyl-7,8-dimethoxyjuglone, m.p. 224–227° (5 mg) suspended in 2 ml EtOH and 2 ml conc HCl was heated at 60–70° for 2 hr. The crystalline ppt was collected after cooling and recrystallized from $CHCl_3$–isooctane to give 3 mg of **20a**, m.p. 246–248°.

[20] See Ref. 17 for reproduction of mass spectrum.

Acid hydrolysis of 2-hydroxy-3-acetyl-5,7,8-trimethoxy-1,4-naphthoquinone (**37**)

The trimethyl ether of spinochrome A (3·5 mg) in 2 ml EtOH and 2 ml conc HCl was heated at 70–80° until the orange soln had turned red. The soln was evaporated *in vacuo* and the residue was chromatographed on a column of acid-treated, deactivated silica gel. Three bands separated upon elution with benzene. The fastest moving band was purple and gave after crystallization from $CHCl_3$–isooctane 1·5 mg of **20a**, m.p. 246–248°. A small amount of 2-hydroxy-7-methoxynaphthazarin, m.p. 240–241°, was obtained from the second band. The third band was yellow and was not investigated.

Sodium borohydride reduction of 2-hydroxy-3-,7-dimethoxy-6-ethylnaphthazarin (**30**)

2-Hydroxy-3,7-dimethoxy-6-ethylnaphthazarin (5 mg) in 10 ml MeOH was treated with 25 mg $NaBH_4$. After standing for 5 min, the mixture was acidified with dil HCl and the product was extracted with ether and transferred into 1N NaOH. After a few sec the aqueous soln was acidified and extracted with benzene. The product was chromatographed on a preparative TLC plate of acid-treated, deactivated silica gel and separated into 4 principal bands using benzene as the developing agent. Band 3 was unchanged starting material while bands 2 and 4 were mixtures of mostly juglones (yellow) and were not investigated. Band 1 proved to be identical in every respect with an authentic sample of 2,6-dimethoxy-7-ethylnaphthazarin.[5]

Sodium borohydride reduction of 2-hydroxy-3-ethyl-6,7-dimethoxynaphthazarin (**29**)

2-Hydroxy-3-ethyl-6,7-dimethoxynaphthazarin (15 mg) in 12 ml MeOH containing a drop 5% NaOH aq was treated with 540 mg $NaBH_4$ over 2 hr. The mixture was acidified with HCl aq and extracted with ether. The products were transferred into dil NaOHaq, the aqueous phase acidified and extracted with benzene, and the benzene extract evaporated. The residue was separated into its components by preparative TLC on acid-treated, deactivated silica gel and 3 principal products were collected. The fastest moving band which was the major product crystallized from isooctane to give *2-hydroxy-3-ethyl-6,7-dimethoxyjuglone* (**14**) as red needles, m.p. 165·5°. A second minor product which travelled slighly slower crystallized from isooctane to yield *2-ethyl-3-hydroxy-6,7-dimethoxyjuglone* (**12**) as orange needles, m.p. 185–187°. The second most abundant product travelled very slowly and proved to be identical in every respect with **48**.

Sodium borohydride reduction of 2,7-dihydroxy-3-acetyl-6-methoxynaphthazarin (**32**)

The monomethyl ether of spinochrome C (1 mg) was dissolved in 5 ml MeOH and treated with ca. 2 mg $NaBH_4$ over a period of 5 min. The product was obtained as described above and chromatographed on preparative TLC using $CHCl_3$ as the developing agent. *2,7-Dihydroxy-3-methoxy-6-ethylnaphthazarin* (**28**), which moved slightly slower than the starting material, was identified by comparison with an authentic sample and by conversion to 2,3,6-trimethoxy-7-ethylnaphthazarin (echinochrome A trimethyl ether) upon methylation with diazomethane.

Sodium borohydride reduction of 2,3,6,7-tetramethoxynaphthazarin

A soln of 10 mg of 2,3,6,7-tetramethoxynaphthazarin (spinochrome E tetramethyl ether) in 25 ml MeOH was treated with excess $NaBH_4$. Aliquots were drawn periodically and acidified and the progress of the reduction was determined by TLC. The mixture was acidified with HCl aq and the major product, *2,3,6,7-tetramethoxyjuglone*, was isolated after preparative TLC, orange–red needles from isooctane, m.p. 140°, UV spectrum in $CHCl_3$: λ_{max} 269, 325, 425 mμ (relative intensities 2·79, 1·06, 0·38).

Acetylation of 2,6-dihydroxy-3,7-dimethoxynaphthazarin and 2,7-dihydroxy-3,6-dimethoxynaphthazarin

Ketene was passed through a suspension of 10 mg 2,6-dihydroxy-3,7-dimethoxynaphthazarin in 20 ml benzene for 10 sec. After 2 hr when the suspended solid had dissolved and the reaction was indicated complete by TLC, the soln was evaporated *in vacuo* and the residual solid was purified by preparative TLC with benzene. The major red band gave 8 mg of *2,6-dimethoxy-3,7-diacetoxynaphthazarin* as red needles from $CHCl_3$–isooctane, m.p. 194–196°. NMR spectrum: C-2 and C-6 methoxyls, δ 4·13; C-3 and C-7 acetoxyls, 2·37; C-5 and C-8 hydroxyls, 12·67. UV spectrum in $CHCl_3$: λ_{max} 310, sh 468, 493, sh 525 mμ (relative intensities 9·6, 6·5, 7·4, 4·9); λ_{min} 370 mμ (relative intensity 1·4).

2,7-Dimethoxy-3,6-diacetoxynaphthazarin was obtained in a similar manner as red needles from $CHCl_3$–isooctane, m.p. 184–185°. NMR spectrum: C-2 and C-7 methoxyls, δ 4·15; C-3 and C-6 acetoxyls, 2·43; C-5 OH, 12·42 or 12·67; C-8 OH, 12·42 or 12·67. UV spectrum in $CHCl_3$: λ_{max} 311, sh 472, 500, sh 532 mμ (relative intensities 8·7, 6·1, 6·9, 4·5); λ_{min} 370 mμ (relative intensity 1·0).