Anal. Calcd for $C_{21}H_{23}NO_4$: C, 71.37; H, 6.56; N, 3.96. Found: C, 71.17; H, 6.68; N, 3.97.

D. (±)-Glaucine (3).—Dehydroglaucine (0.052 g, 0.146 mmol) was reduced with 0.5 g of amalgamated zinc dust in the usual way [see (±)-nuciferine, above] to give *ca.* 0.048 g of (±)-glaucine, obtained as a pale yellow oil, identical in ir, uv, and R_f with an authentic sample of (+)-glaucine. Treating the oil with a solution of 0.030 g of picric acid in 25 ml of ethanol gave 0.061 g (71%) of the crystalline picrate, mp 197–199° dec (lit.[15] mp 193–194°).

(±)-Glaucine *via* the Dehydrohalogenation Route. A. 1-(2′-Bromo-4′,5′-dimethoxyphenyl)-N-(3,4-dimethoxyphenethyl)-acetamide.—A mixture of 13.75 g (0.05 mol) of 2-bromo-3,4-dimethoxyphenylacetic acid,[20] 10.0 g (0.55 mol) of 3,4-dimethoxyphenethylamine, and 100 ml of decalin was refluxed for 4 hr under nitrogen in a Dean–Stark apparatus. On cooling, the reaction mixture deposited a fine white precipitate which was recrystallized from ethanol to give 18.31 g (84%) of the desired phenylacetamide, obtained in two crops, mp 157–158° and 156–158°.

The analytical sample, mp 157.5–158.5°, was recrystallized from ethanol.

Anal. Calcd for $C_{20}H_{24}BrNO_5$: C, 54.80; H, 5.52; Br, 18.23; N, 3.20. Found: C, 54.76; H, 5.44; Br, 18.33; N, 3.22.

B. 1-(6′-Bromoveratryl)-6,7-dimethoxy-3,4-dihydroisoquinoline (12).—The above bromoamide (13.15 g, 0.03 mol) was mixed with 50 g of polyphosphate ester (PPE) and was heated for 18 hr at 95–100°. The reaction mixture was dissolved in 200 ml of tap water, and the resulting clear solution was washed once with ether and made basic with concentrated ammonium hydroxide. Extraction with chloroform, drying the extract over magnesium sulfate, and evaporation to dryness *in vacuo* gave approximately 13.0 g of the crude dihydroisoquinoline (12), which was used in the next step without further purification.

The dihydroisoquinoline 12 was analyzed in the form of its oxalate, a white microcrystalline solid, mp 192–193° (from methanol).

Anal. Calcd for $C_{22}H_{24}BrNO_8$: C, 51.78; H, 4.74; Br, 15.66; N, 2.74. Found: C, 51.57; H, 4.95; Br, 15.72; N, 2.78.

C. 1-(6′-Bromoveratrylidene)-N-carbethoxy-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (9).—The crude dihydroisoquinoline prepared above (12, *ca.* 13.0 g) was dissolved in 200 ml of chloroform and the resulting clear solution was treated with 200 ml of 10% aqueous sodium carbonate solution and 16 ml of ethyl chloroformate while being stirred in a cold water bath at 15–20°. (The chloroformate was added in 2-ml portions during 0.75 hr.) The chloroform layer was separated, washed with dilute hydrochloric acid, dried over magnesium sulfate, and evaporated to give a solid residue. The residue was recrystallized from 1:1 chloroform–absolute ethanol to give the desired product (9), 9.96 g [67%, based on 1-(2′-bromo-4′,5′-dimethoxyphenyl)-N-(3,4-dimethoxyphenethyl)acetamide], mp 218.5–219.5°.

Anal. Calcd for $C_{23}H_{26}BrNO_6$: C, 56.11; H, 5.32; Br, 16.23; N, 2.84. Found: C, 55.96; H, 5.31; Br, 16.52; N, 2.93.

D. N-Carbethoxy-6a,7-dehydronorglaucine (14).—A mixture of the tetrahydroisoquinoline (9, 0.400 g, 0.814 mmol) and 0.5 g of calcium carbonate in 250 ml of anhydrous methanol was irradiated for 14 hr in the usual way. The reaction product was chromatographed twice on silica gel with chloroform [see section B of (±)-Nuciferine *via* the Dehydrogenation Route, above] and was recrystallized from absolute ethanol to give 0.080 g (24%) of N-carbethoxy-6a,7-dehydronorglaucine (14), mp 156–158°.

E. (±)-Glaucine (3).—Dehydroglaucine and (±)-glaucine were prepared exactly as described above under (±)-Glaucine *via* the Oxidative Route.

Registry No.—3, 5630-11-5; 4, 5868-18-8; 6, 22185-92-8; 7, 22185-85-9; 8, 22185-86-0; 9, 22185-87-1; 12 oxalate, 22212-25-5; 13, 13555-30-1; 14, 7630-72-0; 16, 7630-74-2; 17, 22212-26-6; 1-(2′-bromo-4′,5′-dimethoxyphenyl)-N-(3,4-dimethoxyphenethyl)acetamide, 22185-91-7.

Acknowledgment.—We thank the Smith, Kline, and French Laboratories for partial support of this work. One of us (S. C. H.) is indebted to the National Science Foundation for a predoctoral fellowship.

(20) R. D. Haworth and W. H. Perkin, Jr., *J. Chem. Soc.*, 1448 (1925).

The Structures of Herqueinone, Isoherqueinone, and Norherqueinone[1]

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A study of the nmr spectrum of the mold pigment herqueinone has revealed that it is a mixture of two stereoisomers, equilibratable in base. The more stable of these isomers is the substance which previously has been termed isoherqueinone. Mass spectra of many samples of highly purified herqueinone have revealed the persistent presence of variable amounts of desoxyherqueinone, of mass 16 less than herqueinone and having a markedly different fragmentation pattern. Herqueinone and desoxyherqueinone appear to form a charge-transfer complex which is less soluble than herqueinone, and whose separation from herqueinone has not been accomplished. Charge-transfer complexes have also been encountered between norherqueinone and desoxynorherqueinone (atrovenetin). All samples of norherqueinone that have been secured contained large amounts of desoxynorherqueinone, herqueinone, and desoxyherqueinone (mass spectra and nmr spectra). Although all other efforts to demonstrate a structural relationship between herqueinone and norherqueinone have failed, mass spectra have demonstrated the inferred relationship. Mass spectra also afforded evidence that the oxygen-containing ring in herqueinone is attached in the reverse sense to the originally reported structure.

Subsequent to the report[2] of the isolation of the copper-colored pigment herqueinone, preliminary investigations of the pigment were reported simultaneously by two groups of investigators.[3,4] In one of these reports[3] there was also described a second, very high-melting, insoluble red pigment, which could be converted to one of the trimethyl ethers of herqueinone by the same methylation procedure used for herqueinone. In view of this conversion, herqueinone, which contains one methoxyl group, was assumed to be a monomethyl ether of the second pigment. Since the name herqueinone had already been suggested,[5] the second pigment was termed nor-

(1) This investigation was supported in part by a research grant (G 24347) from the National Science Foundation. High-resolution mass spectra were determined on an instrument provided by a grant (GP 5323) from the National Science Foundation.

(2) F. H. Stodola, K. B. Raper, and D. I. Fennell, *Nature*, **167**, 773 (1951).

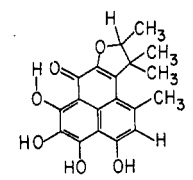
(3) J. A. Galarraga, K. G. Neill, and H. Raistrick, *Biochem. J.*, **61**, 456 (1955).

(4) R. H. Harman, J. Cason, F. H. Stodola, and A. L. Adkins, *J. Org. Chem.*, **20**, 1260 (1955).

(5) Prior correspondence between Stodola, Raistrick, and Cason had resulted in agreement on simultaneous submission of the preliminary investigations, as well as certain details such as nomenclature.

herqueinone. On account of the intractable nature of norherqueinone and the larger yield of purified herqueinone from *Penicillium herquei*, structural investigations have dealt almost exclusively with either herqueinone or the pigment atrovenetin, formed by another organism.[6] Involvement of the last-mentioned pigment resulted from the relationship reported between norherqueinone and atrovenetin.[7] Reduction of norherqueinone with zinc and acetic acid in pyridine gave an amorphous residue containing nitrogen, which was directly acetylated in pyridine to yield in unspecified amount a substance designated as desoxynorherqueinone triacetate. A comparison of numerous physical properties showed this triacetate to be the same substance as atrovenetin triacetate. On the basis of the proposed structure (**1**) for atrovenetin and the relation to herqueinone *via* norherqueinone, it followed reasonably that herqueinone differs from atrovenetin in having an aromatic hydroxyl methylated, and more importantly in having an additional hydroxyl group located in such a manner as to block the fully aromatic system in the atrovenetin structure.

Our investigations of herqueinone concentrated on the alkali-degradable trimethyl ether B, for which structure **2** has been assigned on the basis of chemical and physical evidence.[8] One feature of this structure,



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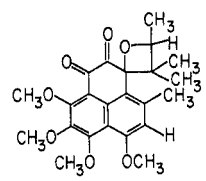

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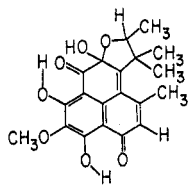

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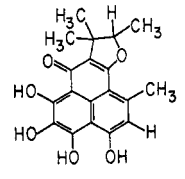
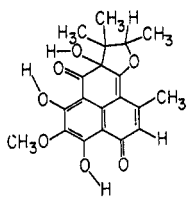


4



5

orientation of the oxygen-containing ring with respect to the side of the naphthalene nucleus bearing methyl, could not be deduced from our investigations, but was assigned on the basis of the reported relationship between herqueinone and atrovenetin.[7] It was also noted[8] that the most reasonable structure for herqueinone, to explain formation of ether B and other derivatives, is that shown in **3**. More recently, an X-ray investigation[9] of the ferrichloride of atrovenetin orange trimethyl ether yielded a structure for atrovenetin differing from structure **1** only in that the oxygen-containing ring was attached in the opposite sense, as in **4**. In view of the above-discussed relationships, it was naturally suggested[9] that herqueinone also contains an oppositely orientated ether ring, and, therefore, is not a hemiketal but a tertiary alcohol and enol ether, structure **5**.[10]

Our investigations, during the course of several years, have failed to establish by chemical methods a structural relationship between norherqueinone and herqueinone, or between norherqueinone and atrovenetin. For example, when a purified sample of norherqueinone was reduced, as has been described,[7] with zinc dust and acetic acid and the amorphous product was acetylated, no homogeneous material could be isolated. This contrasts sharply with the straightforward manner in which herqueinone may be reduced to desoxyherqueinone, and is also in conflict with the report[7] relating atrovenetin and norherqueinone. Methylation of a sample of norherqueinone[11] by the procedure favoring trimethylherqueinone B[8] did, indeed, give trimethylherqueinone B, but in one-tenth of the yield obtained from herqueinone. In the original report[3] of methylation of norherqueinone, there was recorded an 8.7% yield of crude trimethylherqueinone A (yield of pure product not reported[12]), and none of ether B could be isolated. In contrast, methylation of herqueinone gave a 20.7% yield of crude ether A (no report on pure product[12]) and a 6.5% yield of purified ether B.

Also included in the original report of norherqueinone[3] were data on alkali-catalyzed isomerization of both herqueinone and norherqueinone. Whereas the specific optical rotation of herqueinone was reported as 440° and that of isoherqueinone (alkali-isomerization product) as zero,[13] the respective values for norherqueinone and isonorherqueinone were 1080 and −730°. These differences seem remarkable if the two sets of compounds differ only in methylation of a hydroxyl group at a site in the molecule rather remote from the asymmetric centers.

A criterion, independent of norherqueinone, by which herqueinone has been related to atrovenetin[7] involves oxidation of desoxyherqueinone to the same 1,8-naphthalic anhydride obtained by oxidation of atrovenetin. No yields were reported for either conversion. After a series of failures, we eventually succeeded[8] in accomplishing this conversion of herqueinone *via* desoxyherqueinone to the reported anhydride.[7]

(6) K. G. Neill and H. Raistrick, *Biochem. J.*, **65**, 166 (1957).

(7) D. H. R. Barton, P. de Mayo, G. A. Morrison, and H. Raistrick, *Tetrahedron*, **6**, 48 (1959).

(8) J. Cason, J. S. Correia, R. B. Hutchison, and R. F. Porter, *ibid.*, **18**, 839 (1962).

(9) I. C. Paul, G. A. Sim, and G. A. Morrison, *Proc. Chem. Soc.* (London), 352 (1962).

(10) In structure **5**, the oxygen-containing ring is attached on the side adjacent to the methyl group, as has been the designation in previous publications. There is no conclusive evidence on this point; however, it is the case that structure **5** allows better chelation of the hydroxyls than would be possible for the structure resulting from attachment of the oxygen-containing ring on the side adjacent to aromatic hydroxyl. The infrared spectrum of herqueinone[4] shows only chelated hydroxyl. Furthermore, the nmr spectra of herqueinone and isoherqueinone are in support of attachment of the oxygen-containing ring adjacent to aromatic methyl.

(11) R. F. Porter, Ph.D. Dissertation, University of California, Berkeley, 1956.

(12) Our experience has been that the crude precipitate, obtained in the procedure reported[3] for preparation of ether A, consists largely of other compounds; ether A can be separated because of its extreme insolubility and stability at temperatures required for sublimation. A once-crystallized sample gave an analysis for carbon in about 18% error from the calculated value; after this sample had been sublimed, its carbon value was in about 1% error. Our best yields of analytically and spectroscopically pure ether A have been about 2% from herqueinone.

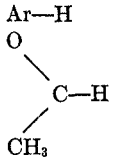
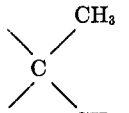
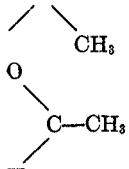
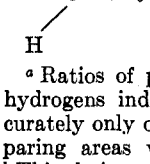
(13) Although we have likewise been unable to observe a rotation for isoherqueinone at the wavelength of the yellow line of sodium, a definite rotation is observable at the blue line of mercury; $[\alpha]^{23}_{Hg} = 200 \pm 100°$.

The yield was about 0.1%. Concern generated by this micro yield was augmented by numerous seemingly impossible inconsistencies, some of which have been mentioned. The only apparent explanation is that one, probably more, of the compounds which had been investigated in our laboratory and in England is a mixture, but extensive efforts at purification failed to demonstrate nonhomogeneity. Application of physical methods to the substances has demonstrated, however, the extensive mixtures that have been under investigation.

The Nature of the Substance Called Herqueinone.—The nmr spectrum of herqueinone (Figure 1) was rather bewildering until it was compared with the spectrum of isoherqueinone, the isomer obtained by heating herqueinone with potassium carbonate in acetone.[3] The multiplicity of peaks in the aliphatic methyl region of the herqueinone spectrum proved to contain one set which corresponds to the peaks in isoherqueinone; indeed, the spectrum of isoherqueinone, which contains only the peaks marked in Figure 1, is readily interpreted (*cf.* Table I) on the basis of the experience

TABLE I

NUCLEAR MAGNETIC RESONANCE DATA

Nature of hydrogens[a]	Peak type	τ Values: Isoherqueinone	Herqueinone[b]	Norherqueinone
Ar—H	Singlet	3.71	3.71	3.71
	Quartet	4.95[c]	5.36[d]	5.35[d]
$Ar—OCH_3$	Singlet	6.14	6.14	*e*
$Ar—CH_3$	Singlet	7.61[f]	7.61	7.60
	Singlet	8.46	8.42	8.41
	Singlet	9.12	8.88	8.91
	Doublet	8.67[c]	8.35[d]	8.35[d]

[a] Ratios of peak areas were in accord with the numbers of hydrogens indicated; however, this could be determined accurately only on the spectrum for pure isoherqueinone, by comparing areas with those of aromatic methyl and methoxyl. [b] This designation refers to the isomer in the herqueinone mixture which is not isoherqueinone. [c] Coupling constant, 6 cps. [d] Coupling constant, 7 cps. [e] The small methoxyl peak (less than one-third the area of the aromatic methyl) was at τ 6.13. [f] In contrast with the peak shown in Figure 1, in the spectrum for isoherqueinone this peak was sharp and symmetrical.

gained in connection with trimethylherqueinone B.[8] Since different samples of herqueinone, isolated from the same batch of mold culture, exhibit different ratios between the two sets of nmr peaks, as in the two tracings in Figure 1, it follows that herqueinone is actually a mixture of stereoisomers which are equilibratable in alkali, and the more stable of these isomers is the substance termed isoherqueinone. Since the peak for hydrogens in the aromatic methyl (τ 7.6) is unsymmetrical on one side or the other in the herqueinone spectra while the peak for methoxyl hydrogens (τ 6.15) is sharp and symmetrical, it follows that the methyl is

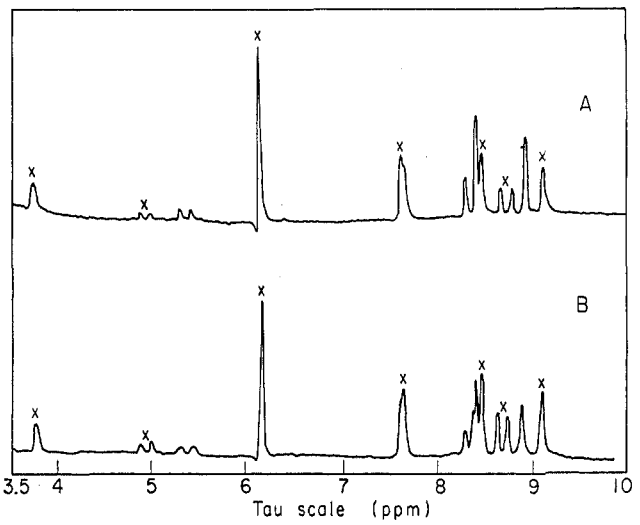
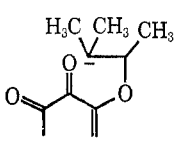


Figure 1.—Nmr spectra of two samples of herqueinone, taken on a Varian A-60 spectrometer, with a 6% solution in pyridine; τ values (ppm) are based on assignment of a value of 10 to tetramethylsilane: curve A, sample crystallized from chloroform; curve B, sample crystallized from ethanol (for details of isolation, *cf.* Experimental Section).

near the region where the herqueinone isomers differ in structure while the methoxyl is not. This is consistent with the oxygen-containing ring being adjacent to the aromatic methyl, as has been proposed. Furthermore, in comparing isoherqueinone and herqueinone, the substantial shift in position of resonance for the methinyl hydrogen, as well as one geminal methyl, indicates that the oxygen-containing ring in the isomers is in a different orientation with respect to the aromatic system. This suggests that the isomers differ with respect to the side of the molecule on which the carbon of the oxygen-containing ring is joined.

In retrospect, the optical rotation of herqueinone isolated in our laboratory has been highly variable, and this is consistent with the presence of two isomers. Rotations reported in the literature are 440[3] and 345°.[4] If herqueinone and isoherqueinone are isomers differing only in stereochemistry at the point of attachment of the aliphatic hydroxyl, then reductive cleavage of this hydroxyl with zinc and acetic acid should give the same compound, desoxyherqueinone, from either isomer. This proves to be the fact (*cf.* Experimental Section). Furthermore, if structure **5** is to be converted to structure **2** (ether B), it seems necessary to assume that anhydrous potassium carbonate in acetone is capable of generating, in small concentration at least, the anion shown in partial structure. Formation of



this anion seems rather remarkable; however, it is no more surprising than conversions encountered in alkaline degradation of ether B.[8] The driving force behind such conversions is reasonably ascribed to relief of steric strain in herqueinone and many of its derivatives. In principle, vinylogous attack of the carbanion on the unsaturated ketone could give either of two stereoisomers; however, the isomer with the

tertiary methyl on the side of the oxygen-containing ring which is opposite that confronted by the aromatic methyl should be subject to much less steric strain. Thus, herqueinone and isoherqueinone should give the same trimethylherqueinone B. This also has been observed.[11]

The mass spectrum of herqueinone shows an abundant molecular ion (*m/e* 372) as well as an ion of *m/e* 356 (M − 16). The ratio of abundances of these two ions varies considerably from one spectrum to another, and may vary severalfold from one instrument to another. Progressive reduction of the ionizing voltage caused the relative abundances of all other ions in the fragmentation pattern to decrease with respect to the ions at *m/e* 356 and 372. At 10–15 V, only the latter two ions remained in the spectrum, and for a short time they remained in the same ratio as observed at 70-V ionizing potential. It is clear that the ion at *m/e* 356 is a molecular ion; therefore, the sample of herqueinone contains significant amounts of a compound with one less oxygen than herqueinone. Examination of eight different samples of herqueinone, prepared from six culture lots, each carefully purified by extraction with bicarbonate, adsorption chromatography, and repeated recrystallization, showed that each sample contained the deoxy compound. Heating of the sample in the mass spectrometer at 200–250° caused selective fading of the ion at *m/e* 372; after this ion was no longer observable, the residual fragmentation pattern was nearly identical with that of pure desoxyherqueinone prepared by reduction of herqueinone with zinc and acetic acid. Thus, desoxyherqueinone is a metabolic product of the mold, but the quantity formed is carried through the purification procedure with herqueinone. This behavior, together with that to be described for norherqueinone, seems explicable only on the basis of a charge-transfer complex between herqueinone (or isoherqueinone, or both) and desoxyherqueinone.

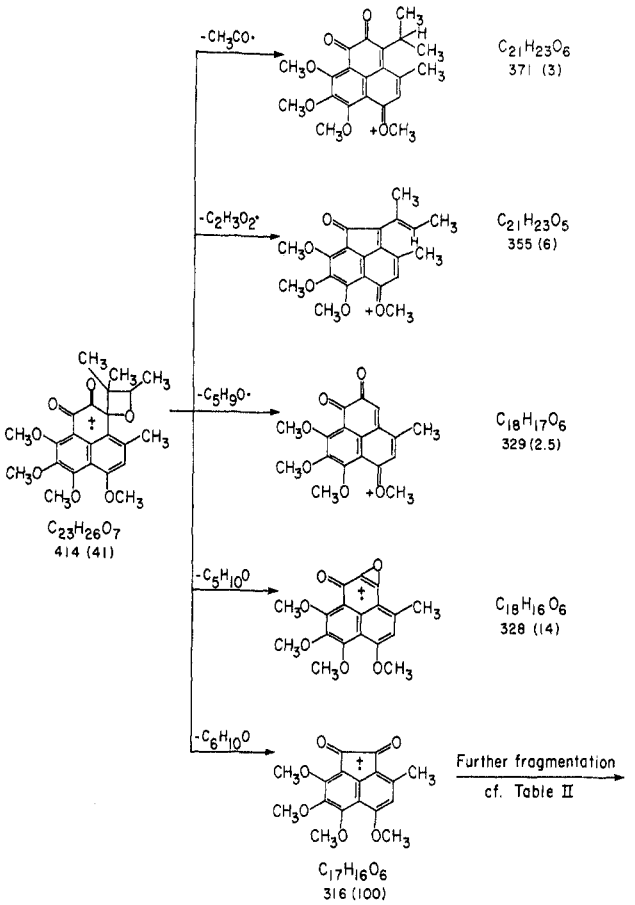
The Nature of the Substance Called Norherqueinone.—Examination of the nmr spectrum (Table I) of a recrystallized sample of norherqueinone (benzene-insoluble pigment) reveals that herqueinone is the most probable source of the herqueinone ethers which have been obtained by methylation of the insoluble pigment called norherqueinone. The sharp peak at τ 6.13 is at the location of hydrogens in the aromatic methoxy in herqueinone (*cf.* Figure 1 and Table I), and no other type of hydrogen found in herqueinone derivatives gives a signal in this region. In addition to the more prominent resonance lines in the norherqueinone spectrum (as listed in Table I), there were weak signals from lines corresponding to the isoherqueinone spectrum: a doublet centered at *ca.* τ 8.67 and a singlet at τ 9.12.

In view of the heterogeneity of our best samples of herqueinone and the occurrence of herqueinone in a purified sample of norherqueinone, six samples of "crude norherqueinone"[14] were examined by mass spectrometry. All samples exhibited molecular ions (verified by reduction of ionizing potential to 10 eV) at *m/e* 356 (desoxyherqueinone) and 372 (herqueinone), but only two samples showed a significant molecular ion at *m/e* 358 (norherqueinone). Both samples showing a molecular ion for norherqueinone also showed molecular ions at *m/e* 342 (desoxynorherqueinone or atrovenetin), 356, and 372; and in only one sample was the norherqueinone ion the most abundant. Surprisingly enough, recrystallization from acetic acid of this best sample failed to alter the content of norherqueinone. Recrystallization increased the relative amount of herqueinone and decreased that of the deoxy compounds.

Since our persistent efforts have failed to yield a sample of norherqueinone not containing significant amounts of atrovenetin (desoxynorherqueinone),[15] conversion of a sample of norherqueinone to a product containing atrovenetin[7] becomes rather dubious evidence that the two pigments are structurally related. This, in turn, leaves in doubt the mode of attachment of the oxygen-containing ring in herqueinone. The nmr spectra (Figure 1, Table I) offer good evidence that herqueinone and norherqueinone do bear the structural relation inferred by the names. Mass spectrometry confirms this relationship, and also supports the same mode of attachment for the oxygen-containing ring as that reported for atrovenetin (structure **4**).

Structural Deductions from Mass Spectra. Trimethylherqueinone B.—The fragmentation pattern of ether

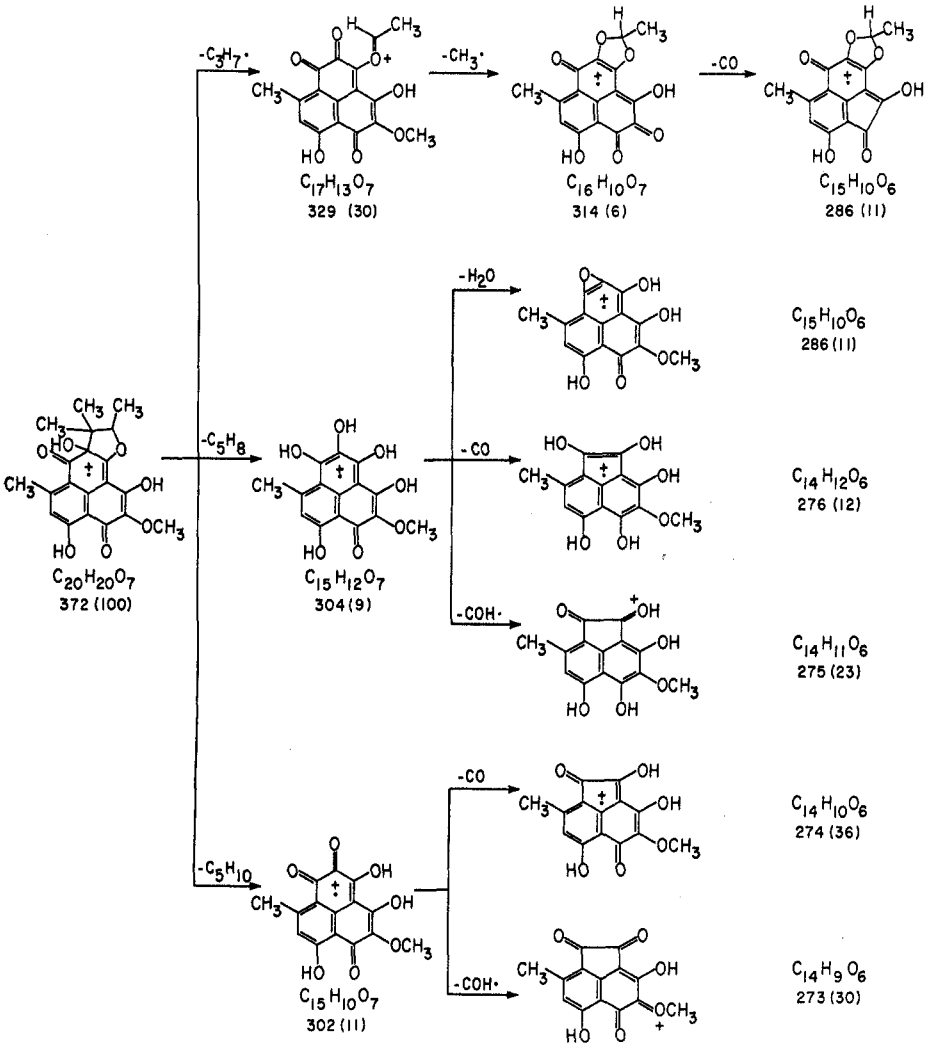
SCHEME I



(14) Norherqueinone has been isolated[3] by crystallization from acetic acid of the insoluble metabolites remaining after extraction of herqueinone with benzene or ether, solvents in which herqueinone is readily soluble; *cf.* Experimental Section.

(15) The only purification of norherqueinone reported[3] by British investigators was recrystallization from acetic acid. Our failure to isolate atrovenetin (desoxynorherqueinone) after reduction of "norherqueinone" may be ascribed to the fact that we used samples of "norherqueinone" which had been recrystallized several times from acetic acid. Such recrystallization reduces the content of atrovenetin in the mixture (*cf.* Chart I).

SCHEME II



B (Tables[16] II and III, Scheme I) offers excellent support for the previously assigned structure[8] for this compound. The principal fragmentation pathway leads to the formation of acenaphthenequinone (*m/e* 316) by loss of the entire spiro ring as a single fragment. The mass spectrum of the synthesized quinone[17] accounts for all ions smaller than *m/e* 316 in the ether B spectrum, which have relative abundances >7% (Table[16] III). In addition, there were only four ions with *m/e* <316 (*m/e* 272, 255, 229, and 215) having abundances >5% which were absent from the quinone spectrum. Another pathway of much less importance involves loss of only three members of the four-atom spiro ring. Since fragmentation from this spirane system would be expected to occur readily, the modest abundances of ions at *m/e* 328 and 329 attest to the great stability of ions possessing the acenaphthenequinone structure. Two other ions of appreciable abundance are at *m/e* 371 and 355. The pathways leading to these fragments are less obvious. The absence from the spectrum of ions corresponding to M − CH_3, M − OCH_3, and M − OCH_2 would seem to preclude fragmentation from the aromatic portion of the molecule. On the other hand, the energetically less stable spiro ring should fragment readily. Possible structures are suggested in Scheme I.

Herqueinone and Isoherqueinone.—Since we have been unable to secure samples of herqueinone or isoherqueinone which are free of desoxyherqueinone, the data for isoherqueinone listed in Table[16] IV resulted after the relative abundances of the ions from a spectrum of pure desoxyherqueinone had been subtracted from the spectrum of isoherqueinone contaminated with desoxyherqueinone. Isoherqueinone and the mixture of isomers termed herqueinone exhibit identical mass spectra.

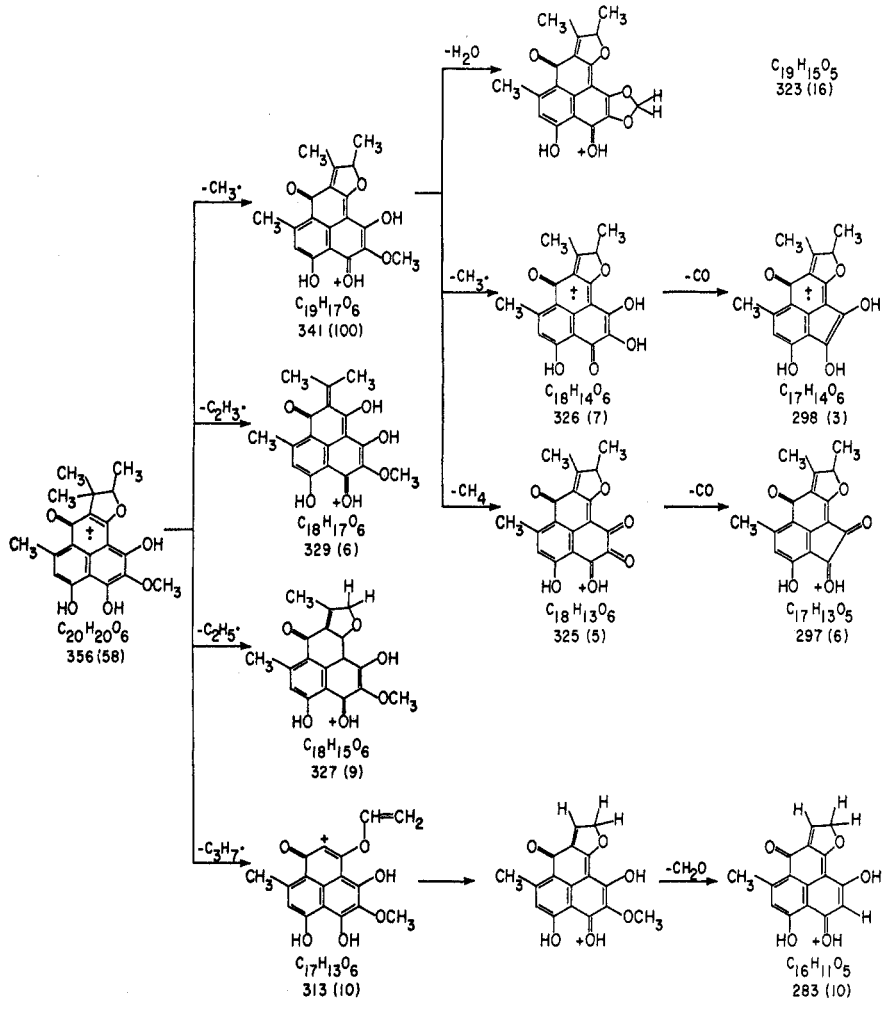
A particularly important feature of the herqueinone fragmentation pattern is the absence of an M − CH_3 peak. This contrasts sharply with the desoxyherqueinone fragmentation (Table[16] V), where M − CH_3 is the base peak. Thus, the hydroxyl in herqueinone must be attached to a quaternary carbon in a manner which blocks interaction between the aromatic system and the *gem*-dimethyl group in the aliphatic ring. Structure **5** has this feature whereas the previously assigned structure **3** does not. Other features of the fragmentations shown in Scheme II support structure **5** rather than the hemiketal structure **3**.

When carbon in the aliphatic ring is attached to the top carbon of the central ring in herqueinone (*cf.* ref

(16) For Tables II–V, order Document NAPS-00572 from ASIS National Auxiliary Publications Service, c/o CCM Information Corp., 909 3rd Ave., New York, N. Y. 10022, remitting $1.00 for microfiche or $3.00 for photocopies.

(17) J. Cason and D. M. Lynch, *J. Org. Chem.*, **31**, 1883 (1966).

SCHEME III



10), the other side of the aliphatic ring may be attached to either flanking oxygen by simple ring opening and closure, with rearrangement of bonds. Since this sort of rearrangement is definitely expected in electron bombardment, the oxygen to which the ring was originally attached cannot be deduced from a consideration of relative ion stabilities in the fragmentation products. If the ion fragments shown in Scheme II are written with the ring oxygen on the side of the aromatic methyl group, the bond structures seem clearly of higher energy than in the analogous ions with the ring oxygen on the side of the aromatic hydroxyl. It is only for that reason that the structures in Scheme II show the ring oxygen on the opposite side from structure **5**.

Although there is a significant degradative route depending on initial loss of hydroxyl to give ions at *m/e* 355, 327 (rel intensity 3), and 311 (*cf.* Table[16] IV), there is no pathway depending on the initial loss of fragments containing two oxygen atoms. Important routes of fragmentation (Scheme II) involve initial loss of hydrocarbon fragments, at *m/e* 329 (M − C_3H_7), 304 (M − C_5H_8), and 302 (M − C_5H_{10}). These fragments can arise only from the aliphatic ring, while *both oxygens* remain attached to the tricyclic system. This feature supports structure **5**.

It is of interest that all but one (*m/e* 286)[18] of the major routes of degradation shown in Scheme II proceed to ions which may be formulated as containing the acenaphthenequinone structure. Further degradation of these ions to the lower mass fragments in Table[16] IV may be described in terms of ions of reasonable structures.

Desoxyherqueinone.—Since the fragmentation of desoxyherqueinone (Table[16] V, Scheme III) is dominated by loss of a methyl group to yield the ion at *m/e* 341, it seems necessary to attach the *gem*-dimethyl grouping to the aromatic system so that a highly stabilized ion results from loss of one of these methyls. Thus, the revised formulation **4** is clearly favored over the initial structure **1**, as was the case in the mass spectrum of herqueinone.

Further degradation of the dominant ion at *m/e* 341 leads to other structures, such as those at *m/e* 297 and 298, which contain the ubiquitous acenaphthenequeinone skeleton. As was the case with ions from herqueinone, lower energy structures result when the ring oxygen is attached on the side of hydroxyl, rather than aromatic methyl.

Another striking difference in the desoxyherqueinone fragmentation is represented by the rearrangement ions at *m/e* 329 (M − C_2H_3), 327 (M − C_2H_5), 313 (M − C_2H_3O), 313 (M − C_3H_7), and 283 (M − C_4H_9O). This entire group of ions may be formulated as arising by initial breaking of the C–C bond adjacent to the ring oxygen followed by random cleavage of one of the

(18) The alternate routes of fragmentation to $C_{15}H_{10}O_6$ (*m/e* 286) are supported by detection of metastable peaks.

TABLE VI

SIGNIFICANT IONS IN THE MASS SPECTRUM OF THE SAMPLE OF BENZENE-INSOLUBLE PIGMENTS CONTAINING THE HIGHEST RATIO OF THE ION OF *m/e* 358 (NORHERQUEINONE)

Ion species	Deoxynorherqueinone *m/e* (%)	Desoxyherqueinone *m/e* (%)	Norherqueinone *m/e* (%)	Herqueinone *m/e* (%)
M	342 (45)	356 (10)	358 (59)	372 (15)
M − CH_3	327 (100)	341 (16)		
M − C_3H_7			315 (80)	329 (8)
M − C_5H_{10}			260 (85)	274 (14)
− CO				
M − C_5H_{10}			259 (14)[a]	273 (8)
− COH				
M after recrystallization	342 (46)	356 (10)	358 (70)	372 (26)

[a] This ion is also a herqueinone fragment, and in this spectrum herqueinone accounts for 6% of the ion of *m/e* 259.

other bonds in the original oxygen-containing ring. A third important difference from herqueinone is that loss of C_5 and C_6 fragments, representing the oxygen-containing ring, is completely absent in desoxyherqueinone. These were the major pathways in herqueinone and ether B. The structure for *m/e* 313 seems the most rational one for the $C_{17}H_{13}O_6$ ion. Further degradation to *m/e* 283 follows a pathway previously observed[19] for loss of methoxyl in an aromatic system.

In all fragmentations of herqueinone and its related ring systems, the aromatic methyl appears to remain intact, while methoxyl is fragmented only by relatively involved routes. In the formation of the ion at *m/e* 283 (Scheme III) there is hydrogen rearrangement; the ion at *m/e* 325 (Scheme III) is formed by concerted loss of methyl from methoxy and hydrogen from hydroxyl. These same degradations occur in other structures examined, as minor pathways. An additional concerted route for methoxyl degradation occurs in formation of the ion at *m/e* 314 (Scheme II); methyl is lost with concurrent ring formation.

Norherqueinone and Desoxynorherqueinone.—Although the two compounds in the nor series have been obtained only in admixture with each other and with herqueinone and desoxyherqueinone, the mass spectrum of the mixture is quite informative. In Table VI are assembled the most prominent and informative ions in the spectrum of the mixture. Ions are conspicuous which correspond to loss of the same fragments from norherqueinone that are lost from herqueinone; indeed, two of the norherqueinone fragments (*m/e* 315 and 260), which represent loss of hydrocarbon moieties, are especially conspicuous. The higher ratio of these ion fragments may well result from a lower stability for the molecular ion in the unmethylated species; if so, this means that the ratio of norherqueinone in the mixture is higher than indicated by the ratio of molecular ions. Since the ions at *m/e* 259 and 260 no doubt arise from loss of COH and CO from the M − C_5H_{10} fragment (*cf.* Scheme II), loss of CO is highly preferred in the unmethylated norherqueinone. Of more interest is the high ratio of the ion at *m/e* 315 in norherqueinone, for the corresponding ion in herqueinone fragments further (Scheme II) by loss of methyl from the methoxy group. Lack of the methyl in norherqueinone prevents this fragmentation, so the M − C_3H_7 ion is of greater abundance. Thus, the mass-spectral evidence is in support of the norherqueinone structure being that inferred from the name.

(19) H. Budzikiewicz, C. Djerassi, and D. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, Inc., New York, N. Y., 1967, pp 237–243.

The only abundant ion fragment in the desoxyherqueinone structure is the dominant M − 15 ion, and the analogous ion (*m/e* 327) is likewise dominant for desoxynorherqueinone. The aromatic character of the ion at *m/e* 342 is further indicated by its stability to heat. When the probe in the spectrometer is heated to about 300°, the molecular ions at *m/e* 372, 358, and 356 disappear within a few minutes, while the ion at *m/e* 342 persists, as does the dominant fragment at *m/e* 327.

Experimental Section

Mass Spectra.—Low-resolution mass spectra, including those where thermal decomposition was studied in the spectrometer, were determined by Miss Sherri Firth using a Varian M-66 instrument. Mass measurements were made by one of us (C. W. K.) on a CEC 21-110B double-focussing high-resolution mass spectrometer.

Isolation of Herqueinone and Norherqueinone.—Methods originally employed[4] for isolation and purification of the pigments have been modified and improved considerably. Culture was in low-form Fernback flasks, with 500 ml of medium per flask (20 g of dextrose, 5 g of Proteose peptone); four flasks were inoculated from a mature potato–dextrose–agar slant prepared from *P. herquei* Bainier and Sartory (Northern Regional Research Laboratory 2249); incubation was in the dark at room temperature (22–28°) for 20–24 days. The mycelium formed a tough mat on the surface. The appearance of different flasks in the same lot sometimes varied considerably, especially in the amount of reddish color on the underside of the mycelial mat and the amount of orange-red pigment accumulating on the bottom of the flask. In addition, higher temperatures appeared to favor "norherqueinone" (benzene-insoluble pigment) at the expense of herqueinone. The mat was broken up by violent shaking, and the total insoluble contents of the flasks were collected on large Büchner funnels containing no paper. After it was pressed under a rubber dam, with vacuum applied, for about 2 days, the mycelium was dried at 50° under vacuum. The filtrate from the mycelium was acidified to pH 1 with concentrated hydrochloric acid and allowed to stand for several days; then the red-brown precipitate was collected by suction filtration and dried.

In a typical run with 36 flasks, the dried mycelial mat consisted of 142 g of green-gray, brittle material with red spots on one side (underside during growth). This material was continuously extracted with ether for 2–3 days in a Soxhlet apparatus; then the marc was removed, dried, ground thoroughly, and returned to the extractor for 2–3 days additional extraction with a new lot of ether. Filtration of the total ether extracts yielded 540 mg of crude "norherqueinone," and extraction of the ether with three 75-ml portions of 5% aqueous $NaHCO_3$ removed 660 mg of material. Evaporation of the ether yielded 8.5 g of crude pigments. Similar treatment of the precipitate filtered from the acidified culture medium yielded 110 mg of crude norherqueinone; extraction with $NaHCO_3$ yielded 210 mg, and 5.0 g was recovered from the ether extract.

The yellow or brown material recovered on acidification of the $NaHCO_3$ extracts yields a compound isomeric with herqueinone and of similar properties (including insolubility in aqueous bicarbonate solution). We have called the material neoherquei-

none; however, considerable investigation has failed to show definitively whether this substance is merely a different ratio of the herqueinone isomers and desoxyherqueinone or contains different types of isomers. The mass spectrum shows the presence of desoxyherqueinone.

For isolation of herqueinone, a 1-g sample of crude product was shaken with 200 ml of benzene for several hours, and the crude "norherqueinone" (250 mg) was removed by filtration. The filtrate was applied to a column of 15 g of Florex XXS, and the benzene was followed by 100 ml of 10% acetone in benzene. The first 25–30 ml of eluate contained most of the red color and yielded 632 mg of red crystalline solid; the remaining eluate yielded 103 mg of dark red solid from which "pure" herqueinone could be obtained only after rechromatography. Two crystallizations from $CHCl_3$ of material from the first eluate yielded 405 mg of soft, copper-colored needles, mp 220–222°. Additional crystallization yields material of mp 222–223° dec, the melting point reported previously[4] for the best material.

For preparation of the samples of herqueinone used for nmr spectra (Figure 1), a sample of mp 222–223° was chromatographed on Florex XXS, and eluted in ten fractions with 3% acetone in benzene. All fractions melted at 222–223°. Fraction 1 was crystallized once from $CHCl_3$, mp 222–223°, and used for curve A, Figure 1. Fractions 5–10 combined were crystallized twice from ethanol, mp 222–223°, and used for curve B, Figure 1.

Norherqueinone used for the nmr spectrum (Table I) was crystallized six times from glacial acetic acid: mp 273–275° (evacuated capillary) (lit.[3] mp 279° dec).

TABLE VII

Desoxynorher-queinone	Desoxyher-queinone	Norher-queinone	Herqueinone
14	47		29
2	10		60
22	68	1	78
21	66	1	85
26	3	6	5

Benzene-Isoluble Pigments.—In addition to the lot of pigments whose spectrum is described in Table VI, five additional lots were examined. For these lots, ratios of peak heights for the molecular ions of interest are shown in Table VII.

Isoherqueinone.—A solution of 100 mg of herqueinone in 10 ml of dry acetone, to which was added 500 mg of anhydrous K_2CO_3, was heated under reflux for 1 hr. After the cooled solution had been diluted with 50 ml of water and acidified, the orange precipitate was collected. Two crystallizations from 95% ethanol yielded 33 mg of orange needles: mp 247.5–250.5° (lit.[3] mp 248–249°); $[\alpha]^{23}D$ 0 ± 30°; $[\alpha]^{23}_{Hg}$ 200 ± 100° (c 0.9 mg/ml, ethanol).

A 10-mg sample of isoherqueinone was dissolved in 2 ml of 1 *N* NaOH and allowed to stand for 5 min, and the pH was reduced to 4. The yellow solid which precipitated turned orange in *ca.* 1 min. The orange solid was collected and crystallized from 95% ethanol to yield 4 mg of orange needles, mp 247–248.5°, no depression on admixture with an authentic sample of isoherqueinone. There was a large depression in melting point on admixture with herqueinone.

The mass spectrum of isoherqueinone was identical with that of herqueinone, and desoxyherqueinone was always present.

Chemical Reduction of Isoherqueinone.—To a solution of 40 mg of isoherqueinone in 5 ml of glacial acetic acid was added 80 mg of zinc dust. After this mixture had been shaken mechanically for 30 min it was filtered into 15 ml of water. The resultant mixture was allowed to stand for 45 min, and the precipitated yellow solid was collected and washed with 10 ml of 1 *N* hydrochloric acid. The dried product (30 mg, mp 120–140°), after one crystallization from acetone and one from benzene, yielded 19 mg of yellow crystals: mp 246–248°; $[\alpha]^{25}D$ 62 ± 10° (*c* 5 mg/ml, acetic acid). There was no depression in melting point on admixture with authentic desoxyherqueinone of mp 246–248°; both uv and ir spectra were likewise identical (lit.[3] for desoxyherqueinone, mp 240–241°; $[\alpha]^{22}D$ 64°).

Registry No.—**4**, 22185-93-9; **5**, 22212-27-7.

A Stereoselective Synthesis of Hydroazulenes

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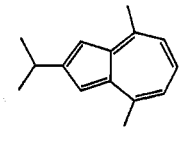
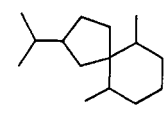
A new synthesis of hydroazulenes based on the cyclization of unsaturated aldehydes is described. The synthesis employs methylated hydrindanones as the starting materials and proceeds *via* solvolytic fragmentation of the corresponding oxime derivatives to the related unsaturated nitriles using *p*-toluenesulfonyl chloride in refluxing pyridine. Reduction to the required aldehyde derivatives was smoothly effected using diisobutylaluminum hydride. Treatment of the aldehydes with silica gel afforded the desired hydroazulenic alcohols.

An interest in the structure and stereochemistry of the vetivane sesquiterpenes prompted us to examine stereoselective synthetic routes to hydroazulenes related to vetivazulene (I).[2] In the course of this work we developed such a route based on the cyclization of unsaturated aldehydes (*e.g.*, **10** → **11**).[3] We subsequently discovered that the vetivane carbon skeleton must be reformulated in terms of the spiro[4.5]decane system II.[4] While this discovery precludes an application of our new hydroazulene synthesis to vetivane sesquiterpenes, we nonetheless present an account of our experimental findings at this time, since (a) the synthetic scheme entails several novel features of intrinsic interest, and (b) applications to other hydroazulenic sesquiterpene types[5] can be envisioned with slight modifications of the scheme.



I



II

The hydrindanone **1**[6] (Scheme I) served as our starting point for these studies. Selective methylation[7] followed by catalytic hydrogenation afforded a mixture of the *cis* and *trans* fused hydrindanones **4c** and

(1) (a) National Science Foundation Predoctoral Fellow, 1964–1966; National Institutes of Health Predoctoral Fellow, 1966–1967; (b) National Institutes of Health Predoctoral Fellow, 1966–1969.

(2) A. St. Pfau and P. A. Plattner, *Helv. Chim. Acta*, **22**, 202 (1939).

(3) J. A. Marshall and N. H. Andersen, *Tetrahedron Lett.*, 1219 (1967).

(4) J. A. Marshall, N. H. Andersen, and P. C. Johnson, *J. Amer. Chem. Soc.*, **89**, 2748 (1967); J. A. Marshall and P. C. Johnson, *ibid.*, **89**, 2750 (1967); *J. Org. Chem.*, **34**, 192 (1969).

(5) *Cf.* P. de Mayo, "Mono- and Sesquiterpenoids," Interscience Publishers, New York, N. Y., 1959, pp 244–276.

(6) G. Stork, A. Brizzolara, H. Landesman, J. Szmuszkovicz, and R. Terrell, *J. Amer. Chem. Soc.*, **85**, 207 (1963).

(7) *Cf.* J. A. Marshall and N. H. Andersen, *J. Org. Chem.*, **31**, 667 (1966).