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# CHEMISTRY OF THE GENUS *SEQUOIA*—III

## STRUCTURAL STUDIES OF ISOSEQUIRIN

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**Abstract**—Recently isolated polyphenolic materials, the sequirins, were subjected to extensive chemical and spectral examination. The skeleton of the sequirins was determined, and subsequently a complete chemical structure of isosequirin was proposed.

### INTRODUCTION

IN OUR recent paper[1] the isolation and characterization of new phenolic compounds, the sequirins, were reported. They were isolated from the extract of the California redwood heartwood (*Sequoia sempervirens*) and were found to be largely responsible for the staining of that wood. Isosequirin, a substance formed upon acidic hydrolysis from naturally occurring sequirin-C and sequirin-B, was the object of our further structural investigations. This sensitive substance can be obtained from the mixture of sequirin-C and -B in yields up to 80 per cent and in high purity. Additional derivatives of isosequirin were prepared to confirm its empirical formula, $C_{17}H_{18}O_5$, which was suggested previously. One of the derivatives, trimethoxy-isosequirin, served as the starting material for various chemical degradations. The examination of the degradation products, in combination with the study of the NMR and mass spectra of the isosequirin derivatives, enabled us to suggest a structure for it.

### RESULTS AND DISCUSSION

*Derivatives of Isosequirin*

The isosequirin pentacetate prepared previously suggested the presence of five free hydroxyl groups. Additional derivatives confirmed that finding. Besides the pentabenzoate and penta-*p*-iodobenzoate (which were of minor importance) the methyl- and methyl-acetyl-isosequirins were prepared. Methylation with dimethyl sulfate yielded a trimethoxy-isosequirin, which upon subsequent acetylation gave a diacetate. Isosequirin thus contains three phenolic and two alcoholic hydroxyl groups and, unlike sequirin-B, apparently no heterocyclic ring. The NMR spectra of these derivatives were in agreement with analytical data. Isosequirin thus contains one alcoholic hydroxyl group more than sequirin-B, but the same number of phenolic hydroxyls. It can be assumed that the rupture of the hetero ring of sequirin-B occurs with a subsequent loss of water and simultaneous rearrangement. The loss of water evidently corresponds to the formation of a new ring, since isosequirin does not contain an olefinic double bond.

[1] B. BALOGH and A. B. ANDERSON, *Phytochem.* **4**, 569 (1965).

*Degradation of Trimethoxy-Isosequirin*

The phenolic materials obtained upon pyrolysis of the sequirins[1] indicated the probable presence of two aromatic rings: one of the catechol type and the other of the *p*-hydroxyphenyl type. However, pyrolysis supplied no evidence regarding linkage of the aromatic groups, and further degradation was needed to complete the elucidation of the skeleton. Isosequirin was also subjected to degradation, but this approach was abandoned after numerous failures. Only in one case—treatment of isosequirin with peracetic acid—could a crystalline carboxylic acid, isosequiric acid, be obtained. It resisted complete purification, and none of its derivatives could be crystallized; therefore it seemed to be unsuitable for further studies. In contrast, trimethoxy-isosequirin, with the phenolic hydroxyls protected, yielded readily isolable degradation products.

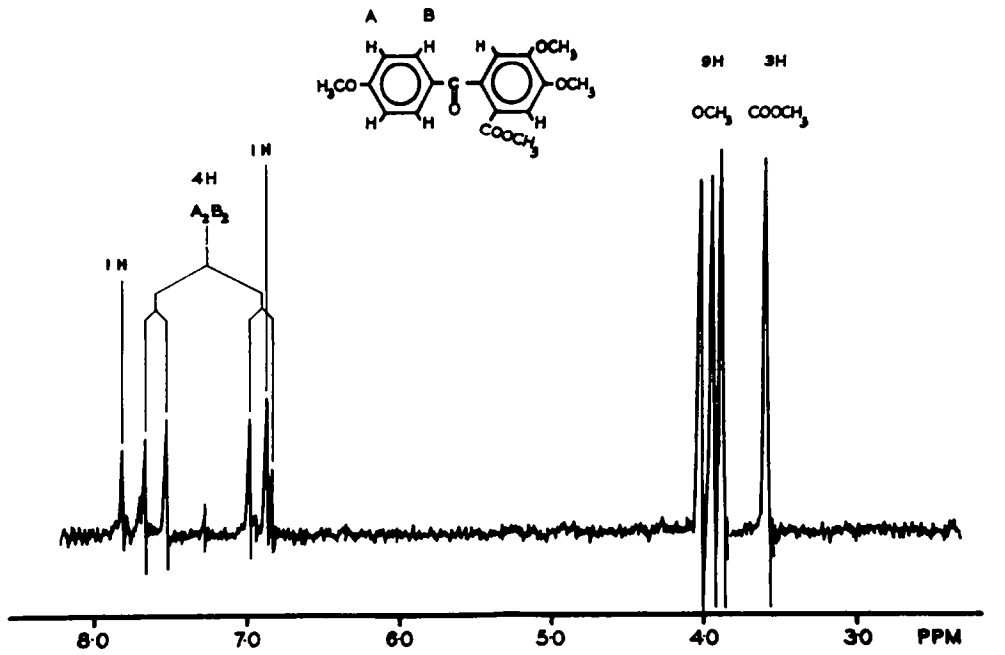



FIG. 1. THE NMR SPECTRUM OF THE MONOMETHYL ESTER (II) OF THE ACID (I) OBTAINED UPON DEGRADATION OF TRIMETHOXY-ISOSEQUIRIN.

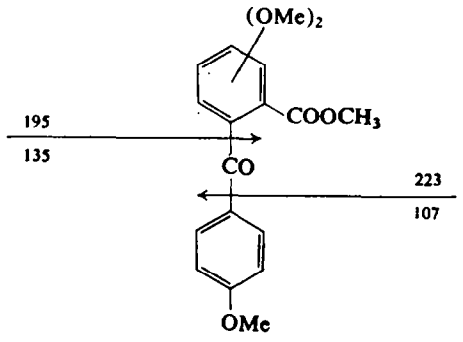
Alkali fusion and degradative oxidations with chromic anhydride (or with $KMnO_4$ in aqueous suspensions at pH 3·7 and 8) yielded products in low yield only, and they could not be identified. Permanganate oxidation in organic solvents such as acetone or pyridine afforded larger amounts of products. In boiling acetone, the oxidation led to a single carboxylic acid (besides some neutral substances, probably polymers) in 10–30 per cent yield. The acid (I) was purified and proved to have the formula $C_{13}H_6(O)(OCH_3)_3COOH$. The three methoxy groups were retained, indicating that the aromatic skeleton suffered no degradation. Methylation of (I) with diazomethane yielded the methyl ester (II) (Fig. 1). The i.r. spectrum indicated that the ester contained no free hydroxyl group. This evidence and the strong absorption maximum of the free acid at 1665 $cm^{-1}$ in conjunction with that at 1710 $cm^{-1}$ suggested a carbonyl group in the vicinity of the carboxyl; therefore a keto acid structure was proposed.

The methyl ester (II) of the NMR spectrum in $CDCl_3$ showed twelve protons belonging to four methyl groups and six aromatic protons (Fig. 1). The three peaks at δ 3·85, 3·96, and 4·04 represent the three phenolic methyl ether groups; the peak at δ 3·6 is due to the ester methyl. Four of the aromatic protons form an $A_2B_2$ system, indicating a *p*-substituted phenyl group. The two other aromatic protons can be assigned to the other aromatic ring.

Since it is unlikely that an α-oxo-acid is obtained upon vigorous oxidation with $KMnO_4$, the carbonyl group present has to be placed between the aromatic rings (the small peak visible at δ 7·3 was caused by the proton contamination of the deuterized chloroform). Thus, the ester (II) is a derivative of benzophenone. Treatment of the keto ester (II) with hydroxylamine provided evidence for the location of the carboxyl group. The reaction product contained no free hydroxyl (i.r. spectrum), and the loss of $CH_3OH$ was indicated by the elemental analysis. (The absence of the ester-methyl protons was also confirmed by the NMR spectrum.) Apparently, the loss of methanol during the treatment resulted in a stable 1,2-oxazine ring, exemplified by the isoxazolone formation from β-keto esters with hydroxylamine.[2] This proved that the ester group is located *ortho* to the carbonyl. Since the aromatic protons on the catechol ring are singlets, the methoxy groups have to be placed between them. With this last observation, the elucidation of the structure of the acid (I) obtained by the degradation of trimethoxyisosequirin was completed. In addition an examination of (II) by mass spectrometry was carried out, from which the principal fragments of rupture are recorded in Table 1.

TABLE 1. PRINCIPAL MASS SPECTROMETRY PEAKS FROM II

| *m/e* | Relative abundance (%) |
|---|---|
| 92 | 27 |
| 107 | 13 |
| 135 | 100 |
| 195 | 2 |
| 223 | 52 |
| 299 | 24 |
| 315 | 7 |
| 330 | 71 |




The fragmentation pattern agrees well with the proposed structure. In addition, there were two metastable peaks detected at masses 85 and 151. They can be related to the following parent–daughter ion-pairs: 85 to (135 → 107) and 151 to (330 → 223), both pairs produced by decomposition of the parent ions.

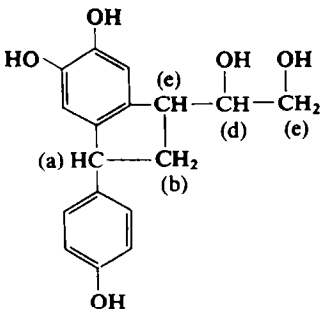
[2] A. HANTZSCH, *Ber. Deut. Chem. Ges.* **24**, 497 (1891); R. E. ROSE and W. SCOTT, *J. Am. Chem. Soc.* **39**, 278 (1917).

*The Structure of Isosequirin*

To complete the structure of isosequirin, the residual aliphatic fragment $C_3H_7(OH)_2$ has to be attached to the aromatic diphenylmethane skeleton determined by the preceding data. The nature of the two alcoholic hydroxyls was recognized by oxidation tests with either periodic acid or lead tetracetate. Formaldehyde was produced, indicating the presence of a hydroxymethylene group in a vicinal glycol arrangement of the hydroxyls. No other oxidation products could be isolated from the oxidation mixtures.

Fragmentation studies by mass spectrometry conducted on isosequirin itself and on trimethoxyisosequirin provided the exact numerical molecular weights of 302 and 344, respectively. Furthermore, the most intense peaks ("base peaks") were observed at M-61 position in both spectra (241 and 283). This loss of mass 61 evidently originated upon rupture of the aliphatic part of the molecules and represents the fragment $C_2H_5O_2$. Due to the absence of methyl groups in isosequirin itself, the fragment $C_2H_5O_2$ can be assigned to a vicinal glycol chain only: —CH(OH)—$CH_2$—OH. This result completes the picture obtained from the oxidation tests. A detailed examination of the fragmentation shows the presence of a *p*-hydroxyphenyl group; further rational interpretation of the fragmentation, however, was less satisfactory.

All preceding data presented in this paper suggest that isosequirin is a derivative of diphenylmethane with a condensed alicyclic ring bearing a vicinal glycol chain, probably adjacent to the catechol group (III).




(III)

The NMR spectra of isosequirin derivatives support this structure. From an examination of Fig. 2, in which the locations of the absorption peaks of the seven aliphatic protons are recorded (excluding absorptions of protons in methoxy and acetyl groups), the following assignments can be made: The $2H_b$ showing a multiplet represents a methylene group: —$CH_2$—. $H_c$ appearing as a triplet in all spectra ($J_{bc}=7{\cdot}9$ c/s) is split a second time ($J_{cd}=2{\cdot}5$ c/s) and can be the italicized proton in the fragment —$CH_2$—C*H*—CH—. $H_a$, the second triplet ($J_{ab}=8$ c/s), can be assigned to a single proton adjacent to the methylene group: —C*H*—$CH_2$—. The absorption peaks which shift upon acetylation must be assigned to the protons attached to hydroxyl-bearing carbon atoms, $H_d$ and $2H_e$.

The sequence =CH—$CH_2$—CH—CH(OH)—$CH_2$—OH is the final result, which, if fitted
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into the determined skeleton, results in (III), as the structure of isosequirin.

The investigation to elucidate the structures of the other sequirins continues.

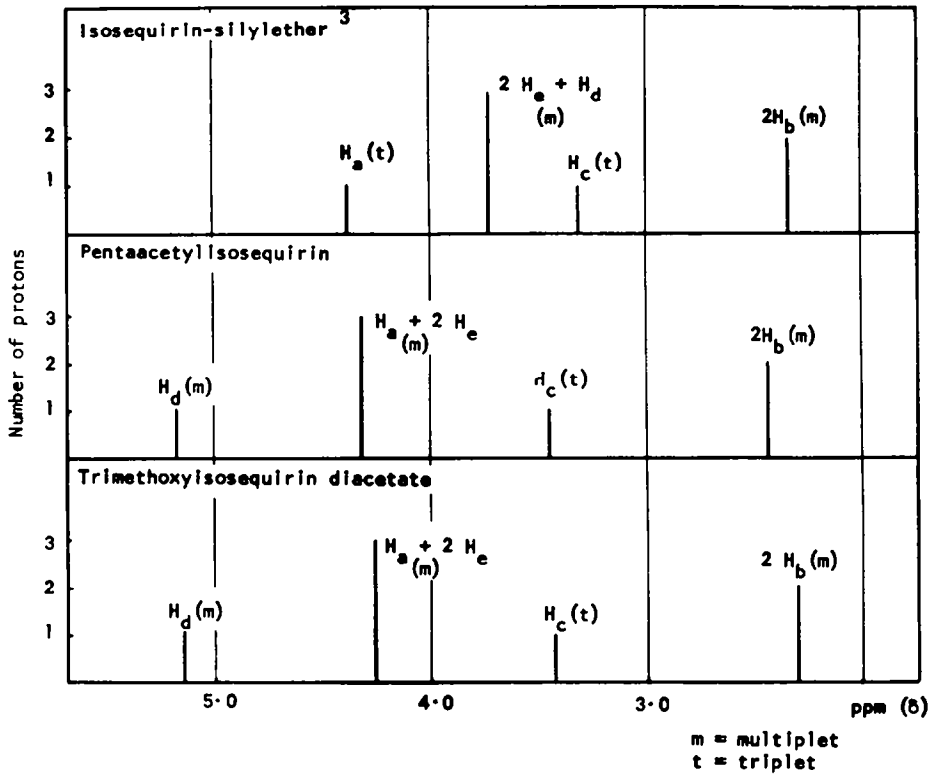


FIG. 2.

## EXPERIMENTAL

Infrared spectra were determined on a Perkin-Elmer Model 21 spectrophotometer (the compounds were embedded in KBr pellets), NMR spectra on a Varian A-60 using tetramethylsilane (TMS) ($\delta = 0$) as internal standard, and mass spectra on a mass-spectrometer, Type 21-103 C (Consolidated Electrodynamic Co., Pasadena, California). Microanalyses were made by Alfred Bernhard, at the Max-Planck-Institut für Kohlenforschung, Mülhelm, Germany.

*Isosequirin pentabenzoate.* Prepared with benzoyl chloride in pyridine at $-5°$. Long, colorless needles from methanol, m.p. 142°. Calc. for $C_{17}H_{13}O_5(C_6H_5CO)_5$ (822·8): C, 76·0; H, 4·66: Found, C, 75·87, 76·05; H, 4·65, 4·80; mol. wt. 765, 781 (acc. to Rast).

*Isosequirin penta-(p-iodo-benzoate).* This was obtained after 24 hr standing of a mixture of isosequirin and *p*-iodo-benzoychloride in pyridine. Long, colorless, thin needles from benzene–abs. alcohol (2:1), m.p. 232°. Calc. for $C_{17}H_{13}O_5(C_6H_4ICO)_5$ (1452·4): C, 43·02; H, 2·29; I 43·7; Found: C, 43·06, 43·05; H, 2·18, 2·00; I, 42·81, 39·98; mol. wt. 1511, 1550 (acc. to Rast).

*Trimethoxy-isosequirin.* By treatment of isosequirin with dimethylsulfate under hydrogen. Opaque, light needles, from benzene, m.p. 119°, which darken upon long exposure to light. Calc. for $C_{17}H_{15}O_2(OCH_3)_3$(344·4): C, 70·32; H, 7·03; ($OCH_3$), 27·07; Found: C, 69·92, 69·87; H, 7·01, 7·11; $OCH_3$, 26·88, 26·87; mol. wt. 342, 347 (acc. to Rast).

[3] Preparation according to: A. C. WAISS, Jr., R. E. LUNDIN and D. F. STERN, *Tetrahedron Letters* **10**, 513 (1964).

In its NMR spectrum, the 3-methoxy proton peaks are located at δ 3·73, 3·79, and 3·87.

*Trimethoxy-isosequirin diacetate.* Via acetic anhydride and sodium acetate from trimethoxy-isosequirin. Colorless, light needles from alcohol, m.p. 114°. Calc. for $C_{17}H_{13}O_2(OCH_3)_3(CH_3CO)_2$ (428·5): C, 67·3; H, 6·59; $CH_3O$, 21·7; Ac, 20·08; Found: C, 67·22, 67·27; H, 6·65, 6·64; $CH_3O$, 21·48, 21·5; Ac, 19·87, 19·80; mol. wt. 437, 441 (acc. to Rast).

In its NMR spectrum, methoxy proton absorptions are at δ 3·74, 3·82, and 3·89; two acetyl proton peaks at δ 2·07 and 2·10.

*Isosequiric acid.* A suspension of isosequirin (1 mole) in water was treated with technical (40 per cent) peracetic acid (2 moles) until all isosequirin had dissolved. Upon evaporation of the water, light yellow octahedric crystals were obtained. Recrystallization from water yielded nearly colorless octahedric crystals, m.p. 218° (decomp.). Calc. for $C_{15}H_{16}O_3(COOH)_2$ (334·3): C, 61·1; H, 5·43; acid equivalent 167·1; Found: C, 60·99; H, 5·61; acid equivalent 187.

*Oxidation of trimethoxy-isosequirin with* $KMnO_4$. A 0·5 g portion of trimethoxy-isosequirin was dissolved in 25 ml of acetone and refluxed with 0·75 g of pulverized $KMnO_4$. After standing overnight, the acetone was removed by evaporation, and the residue was taken up with water and treated with $SO_2$. The oily precipitate obtained was extracted with ether. The acidic fraction containing one acid (I) only was separated with $NaHCO_3$ solution. Recrystallization of the acid from $CHCl_3$-light petrol yielded thin, colorless needles, m.p. 195°. Calc. for $C_{13}H_6O(OCH_3)COOH$ (316·3): C, 64·6; H, 5·10; $CH_3O$, 29·4; Found: C, 64·55, 64·41; H, 5·15, 5·12; $CH_3O$, 28·99, 29·28; ml. wt. 320, 320 (acc. to Rast).

*Methylation of the acid* (I). The acid (I) treated with excess of diazomethane in ether afforded the monomethyl ester (II), glittering, colorless prisms from ethanol, m.p. 152°. Calc. for $C_{13}H_6O(CH_3O)_3COOCH_3$ (330·3): C, 65·6; H, 5·5; $CH_3O$, 37·6; Found: C, 65·42, 65·42; H, 5·51, 5·35; $CH_3O$, 37·4, 37·44.

*Treatment of the ester* (II) *with hydroxylamine.* A 0·05 g portion of the ester (II) was heated with a mixture of 0·05 g of hydroxylamine hydrochloride, 0·25 ml of pyridine and 0·25 ml of abs. alcohol in a sealed tube for 2 hr at 105°. Upon cooling, a white precipitate was obtained. The solvents were removed in vacuum at 60° and the residue washed with hot water. Weight: 0·048 g. Microscopic prisms from hot methanol, m.p. 217°. Calc. for $C_{13}H_6(OCH_3)_3COON$ (313·3): C, 65·2; H, 4·8; N, 4·48; $OCH_3$, 29·7; Found: C, 65·16, 65·04; H, 4·83, 4·87; N, 4·52; $OCH_3$, 29·37, 29·49.

The NMR spectrum showed only three methoxy-proton absorption peaks at δ 3·95, 4·04, and 4·16.

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