Phytochemistry, 1976, Vol. 15, pp. 1285–1287 Pergamon Press. Printed in England.

NEW NORLIGNANS OF *SEQUOIADENDRON GIGANTEA*; PHYTOCHEMICAL COMPARISON WITH *SEQUOIA SEMPERVIRENS*

P. HENLEY-SMITH and D. A. WHITING
Department of Chemistry, The University of Nottingham, NG7 2RD, England

(*Received* 14 *January* 1976)

Key Word Index—*Sequoiadendron gigantea*; *Sequoia sempervirens*; Taxodiaceae; sequirin; norlignan.

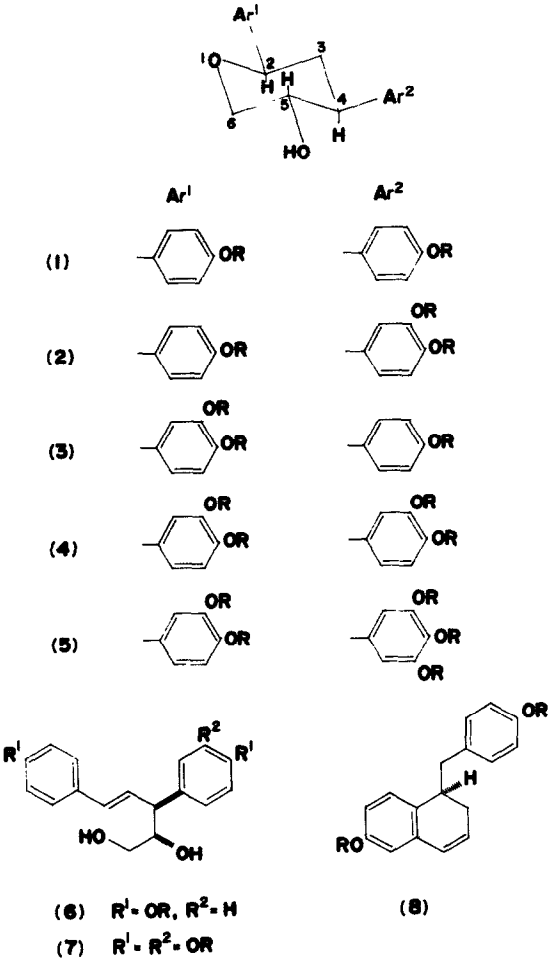
Abstract—The permethyl ethers of three new norlignans (sequirins-E, -F, and -G) from *Sequoiadendron gigantea* heartwood have been characterized by NMR and MS as 2-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl-, 2,4-(3,4-dimethoxyphenyl)-, and 2-(3,4-dimethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-5-hydroxytetrahydropyran respectively, with the 2,5-*trans*,4,5-*trans* stereochemistry. Sequirins A-D, characteristic norlignans of *Sequoia sempervirens* Endl., could not be detected in *S. gigantea* heartwood, nor could sequirins E-G be found in *S. sempervirens* heartwood. Agatharesinol was a common constituent.

INTRODUCTION

Sequoia sempervirens (D. Don) Endl. and *Sequoiadendron gigantea* Lindl. (the "Big Tree" of California) are closely related, previously grouped together as the two species of the genus *Sequoia*, but presently classified in separate monotypic genera. The phenolic constituents of *S. sempervirens* heartwood have been investigated [2] and include sequirins A (1; R=H), B (2; R=H), C (7; R=H), and D (8; R=H), agatharesinol (6; R=H), and some simpler phenols (Table 1). The sequirins are of interest as unusual biogenetic variants of phenylpropanoid metabolism, and the phenolic compounds may contribute to the fungal resistance of the wood. In this paper we report on the phenolic constituents of *S. gigantea* heartwood, propose constitutions for three new norlignans found therein, and compare the natural phenols identified so far in the two heartwoods.

RESULTS

Heartwood shavings of *S. gigantea** were extracted with hot water, closely following previous methods [2a] and the aqueous concentrate exhaustively extracted with ether to provide the phenol-containing fraction. No pure phenols could be separated in the free state, from this mixture, which was then fully methylated. Batch separation of component methyl ethers was then achieved by column chromatography, and individuals purified by repeated TLC. At least 17 components were present, 8 only in trace quantities; none of these was a sequirin A-D methyl ether. Dimethyl agatharesinol [3], dimethyl agatharesinol acetonide, veratraldehyde, and methyl anisate were identified by comparison with authentic specimens. Two constituents (also found in *S. sempervirens*) remain unidentified. The remaining 3 constituents proved to be the permethyl ethers of new nor-lignans related to sequirins-A and -B, and referred to as sequirins -E, -F, and -G. These methyl ethers have been assigned structures (3; R=Me), (4; R=Me), and (5; R=Me) respect-

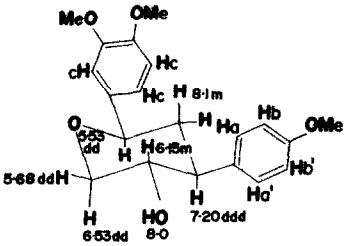


* Specimen collected at Radyr, South Wales. We thank Dr. W. M. L. Crombie for botanical identification.

Table 1. Phenolic heartwood constituents of *Sequoia* and *Sequoiadendron*

Components of methylated phenolic extract	*S. sempervirens*	*S. gigantea*
Dimethylsequirin (1; R=Me)	+	−
Trimethylsequirin B (2; R=Me)	+	−
Trimethylsequirin C (7; R=Me)	+	−
Dimethylsequirin D (8; R=Me)	+	−
Trimethylsequirin E (3; R=Me)	−	+
Tetramethylsequirin F (4; R=Me)	−	+
Pentamethylsequirin G (5; R=Me)	−	+
Dimethylagatharesinol (6; R=Me)	+	+
Methyl anisate	+	+
Anisaldehyde	+	−
Veratraldehyde	−	+
p-Dimethoxybenzene	+	+
(Two unidentified ethers)	+	+

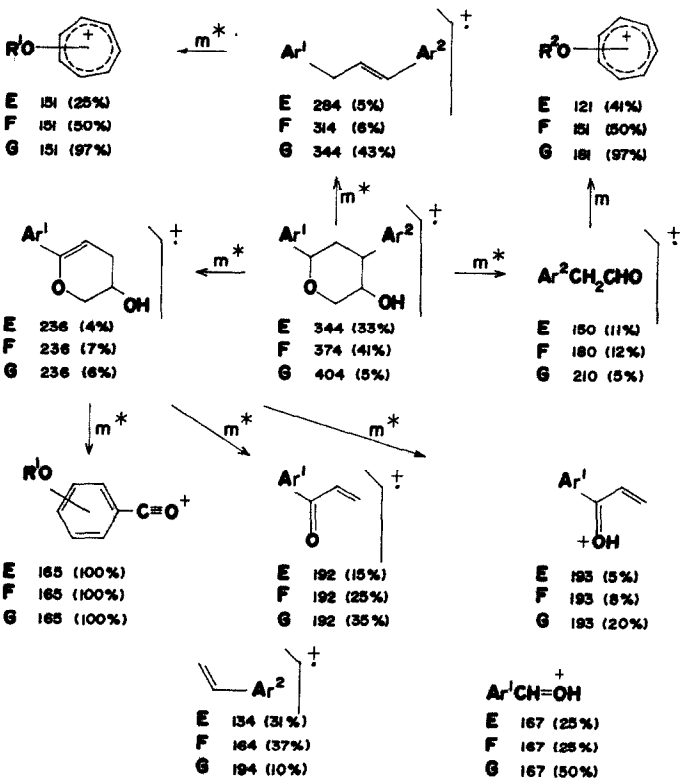
ively. The parent phenols (3; R=H), (4; R=H), and (5; R=H) are presumed to be the true natural products, since ^{1}HMR examination of the extract before methylation showed no methoxyl proton signals. The NMR test, using the PFT technique, readily showed the methoxyl signal from trimethylsequirin B added to the extract at 0.3%, i.e. below the level of sequirins-E, -F or -G actually present (0.3–0.6%).



$J_{2,3ax}$	10	$J_{4,5}$	10	Haa'	2·77d (J 9 Hz)
$J_{2,3eq}$	3	$J_{5,6eq}$	4	Hbb'	3·10d (J 9 Hz)
$J_{4,3ax}$	10	$J_{5,6ax}$	10	Hc	3·14 br s
$J_{4,3eq}$	4	$J_{6,6}$	11	CH_3O	6·10s, 6·14s, 6·20s

^{1}HMR (τ, $CDCl_3$; J in Hz) data for trimethylsequirin-E.

Trimethyl sequirin-E, $C_{20}H_{24}O_5$ isomeric with trimethyl sequirin-B) was obtained as crystals, mp 123.5–125°. Structure (3; R=Me) was indicated by NMR and MS. The assignments for the ^{1}HMR spectrum are shown in the Figure and are definitive of the 2,4-diaryl-5-hydroxytetrahydropyran structure. The tabulated coupling constants indicate the 2,4,5-*eq* chair geometry. The 1,4-substitution pattern of one aromatic ring is apparent from the data; the 3 H of the other are nearly shift-equivalent, as observed for the dimethoxyphenyl of trimethylsequirin



Scheme. Generalized electron impact fragmentation pattern of sequirins [2a,4]. Appropriate metastable ions (M*) have been observed in one or more examples. Percentage abundance (base peak 100%) is given in parentheses. F = tetramethylsequirin F, E = trimmethylsequirin E, G = trimethylsequirin G; $-OR^1$ relates to Ar^1, OR^2 relates to Ar^2.

B. The placing of the aryl rings as 2-(3,4-dimethoxyphenyl)-4-(4-methoxy-phenyl), (implicit in the isomerism with trimethylsequirin-B), is shown by the mass spectrum. The Scheme shows a generalized fragmentation pattern for 2,4-diaryl-5-hydroxytetrahydropyrans derived from data from sequirin B [2a] and sequirin A [4]; trimethylsequirin-E fits clearly into this pattern, which clearly differentiates the aryl rings. These deductions have been confirmed by the total synthesis of (±)-trimethylsequirin E [5], chromatographically and spectroscopically identical with the natural material.

The MS fragmentation scheme was particularly valuable in assigning structures to tetramethylsequirin F ($C_{21}H_{26}O_6$) and pentamethylsequirin G($C_{22}H_{28}O_7$). Only small samples could be obtained and neither could be crystallized. However, their electron-impact fragmentations fit comfortably into the Scheme for 5-hydroxytetrahydropyrans, with the first-named compound possessing $Ar_1 = Ar_2$ = dimethoxyphenyl, and the second having Ar_1 = dimethoxyphenyl Ar_2 = trimethoxyphenyl. Their NMR spectra are also consistent with these structures, the non-aromatic H pattern being similar to that for sequirin E (although obtained for pentamethylsequirin G with poorer resolution), and aromatic and methoxyl protons in the correct ratio. The aromatic substitution pattern for these compounds cannot be demonstrated by NMR since the aromatic protons are close in chemical shift and insufficient material for other tests was available. However, since both rings are shikimate-derived, there must be a very strong biogenetic presumption in favour of the patterns [4] and [5], which are so characteristic of phenylpropanoids.

Parallel with this study, we re-examined *S. sempervirens* heartwood, using the same extraction procedure and exhaustive TLC of the methylated phenols: none of the compounds (**3**; R=Me), **4**; R=Me), or (**5**; R=Me) could be found. Table 1 shows the phenol methyl ethers isolated from the two heartwoods by the same methods. An interesting phytochemical distinction emerges, with agatharesinol (**6**; R=H) as the only common norlignan component.

EXPERIMENTAL

Extraction of Sequoiadendron gigantea *heartwood.* Heartwood (500 g) was reduced to fine shavings and steeped in water (4 dm^3) at 50° for 12 hr; after filtration of the extract, water soaking was repeated at 50° for 12 hr and ambient temperature for several days. The whole aqueous extract was concentrated to *ca* 300 cm^3 and extracted with ether (12 × 200 cm^3). The dried ether extracts were evaporated and the residual gum (5.9 g) dissolved in Me_2CO (75 cm^3). This solution was refluxed with MeI (20 cm^3) over anhyd K_2CO_3 (25 g) for 6 hr; further MeI (10 cm^3) was added in portions during this time. The cooled product was diluted with water and extracted with ether; the ethereal extracts were dried and evaporated to give a brown oil containing the phenyl methyl ethers. The mixture was chromatographed on a silica gel column with C_6H_6–Me_2CO gradient elution (initially 12:1, increasing acetone ratio to 6:1). Fractions were monitored by TLC and bulked into batches with 4–7 components each. The methyl ethers of sequirins -F, -G, and -E were eluted 3rd, 5th, and 6th from the column and were purified by repeated PLC using Si gel HF254 (Merck/Stahl) in 1-mm layers. Tetramethylsequirin -F was obtained as a clear gum, which failed to crystallize, using (a) C_6H_6–Me_2CO (5:4) and (b) $CHCl_3$, (M^+ 374.170. $C_{21}H_{26}O_6$ requires M^+ 374.173). Pentamethylsequirin -G, also non-crystalline, was purified with (a) EtOAc–petrol (bp 40–60°) (4:1) and (b) $CHCl_3$ (M^+ 404.183. $C_{22}H_{28}O_9$ requires 404.184). Trimethylsequirin-E was purified with (a) EtOAc–petrol (b.p. 40–60°) (4:1) and (b) $CHCl_3$, and gave crystals, mp 123.5–125° from C_6H_6 (Found: C, 69.5; H, 6.8%. M^+ 344.161. $C_{20}H_{24}O_5$ requires C, 69.75; H, 6.95%. M^+ 344.162).

REFERENCES

1. '*A Handbook of Coniferae and Ginkgoaceae*', W. Dallimore and A. B. Jackson., 4th Edition revised S. G. Harrison, London, 1966.
2. (a) Hatam, N. A. R. and Whiting, D. A. (1968) *J. Chem. Soc.* (*C*), 1921; (b) Riffer, R. and Anderson, A. B. (1967) *Phytochemistry* **6**, 1557; (c) Begley, M. J., Davies, R. V., Henley-Smith, P. and Whiting, D. A. (1973) *Chem. Commun.* 649.
3. Enzell, C. R. and Thomas, B. R. (1965) *Acta Chem. Scand.* **19**, 913.
4. Enzell, C. R., Thomas, B. R. and Wahlberg, I. (1967) *Tetrahedron Letters* 2211.
5. Paez B. A. and Whiting, D. A. (unpublished work).