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# LIGNANS AND RELATED PHENOLS—X

## ASSIGNMENT OF STRUCTURE TO THE PRINCIPAL CLASSES OF LIGNANS BY GAS CHROMATOGRAPHY

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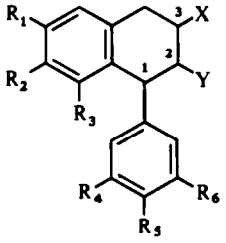
**Abstract**—Gas chromatography as applied to the separation of lignans is described for the first time. The principal classes can be differentiated and geometrical isomers may usually be distinguished.

IN RECENT years a substantial number of new lignans have been isolated from a growing number of natural sources,[1] yet the biogenetic equivalents of many known structures remain undetected *in vivo*: a description of the gas chromatography of these compounds is therefore timely. The method was developed in conjunction with work on the biogenetic-type synthesis of lignans, where mixtures containing more than one of the main classes may occur. Within a given class resolution of isomers could well be improved by use of a more polar stationary phase, although "tailing" of the eluent would soon impose a limitation. The free OH group of tertiary alcohols does not seriously impair resolution, hindered phenols having syringyl substituents may also give acceptable results; we have otherwise confined our study to those compounds where polar groups can be blocked by methylation. The gas chromatography of the trimethylsilyl ethers of related phenols of lower mol wt has been described[2] and details of a procedure for the complete methylation of phenols has also been published.[3]

Reference to the Tables shows that members of the aryltetrahydronaphthalene class (Table 1, X, Y = Me) have the shortest retention times ($t_r$). Here the chromatogram responds sharply to a difference in configuration, for isogalbulin (I) with the more compressed *cis*-configuration has a lower $t_r$ value than galbulin (II). The retention time is also reduced for otobain (III) as compared with its geometrical equivalent, galbulin, a difference which must arise in part from the lower mol wt of the bis-methylenedioxy compound. In methylotobaphenol (V), where the A ring retains two OMe groups, the mol wt difference has had only a small effect on retention and this is evidence of a polar factor. It is to be expected that a retained 3,4-dimethoxyphenyl group would largely determine $t_r$ because it is more nucleophilic than the methylenedioxy analogue; the greater rate of bromination and of acid-catalysed deuterium exchange[4] in veratrole as compared with methylenedioxy benzene is relevant here. It will be seen that in furanoid lignans (Table 3) there is again a marked reduction in $t_r$ when *both* aromatic rings have the less polar methylenedioxy substituent. As the great majority of lignans have two identical oxyaryl groups we have not been able to adduce as much evidence of this dominant polar factor as we would

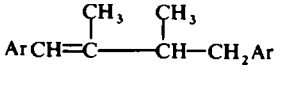
TABLE 1. ARYLTETRAHYDRONAPHTHALENE DERIVATIVES



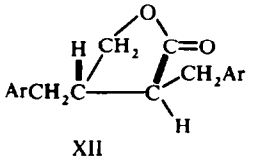
| Compound | Substituents | | | | | | | | Configuration | | $t_r$ relative to cholestane |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | X | Y | C1–C2 | C2–C3 | |
| I Isogalbulin | MeO | MeO | H | H | MeO | MeO | $CH_3$ | $CH_3$ | *cis* | *trans* | 0·21 |
| II Galbulin | MeO | MeO | H | H | MeO | MeO | $CH_3$ | $CH_3$ | *trans* | *trans* | 0·53 |
| III Otobain | H | O—$CH_2$—O | | H | O—$CH_2$—O | | $CH_3$ | $CH_3$ | *trans* | *trans* | 0·39 |
| IV 1β-Hydroxyotobain* | H | O—$CH_2$—O | | H | O—$CH_2$—O | | $CH_3$ | $CH_3$ | *trans* | *trans* | 0·52 |
| V Methylotobaphenol | MeO | MeO | H | H | O—$CH_2$—O | | $CH_3$ | $CH_3$ | *trans* | *trans* | 0·51 |
| VI Desoxypodophyllotoxin | O—$CH_2$—O | | H | MeO | MeO | MeO | $CH_2$—O—CO | | *cis* | *trans* | 2·42 |
| VII Desoxypricropodophyllin | O—$CH_2$—O | | H | MeO | MeO | MeO | $CH_2$—O—CO | | *trans* | *cis* | 2·68 |
| VIII Dimethyl α-Conidendrin | MeO | MeO | H | H | MeO | MeO | CO—O—$CH_2$ | | *trans* | *trans* | 2·15 |
| IX Dimethyl β-Conidendrin | MeO | MeO | H | H | MeO | MeO | CO—O—$CH_2$ | | *trans* | *cis* | 2·65 |

* The OH group is *cis* to C2—$CH_3$ this $t_r$ and that of dehydro-otobain (0·33) was obtained at 230°.

TABLE 2. ACYCLIC LIGNANS



X



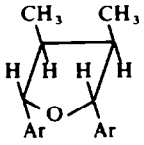
XII

| Compound | Ar Substituent | $t_r$ relative to cholestane |
|---|---|---|
| X Dimethylguaiaretic acid | 3,4-methoxyphenyl | 0·68 |
| XI *meso*-Dihydro derivative | 3,4-methoxyphenyl | 0·56 |
| XII Dimethylmatairesinol | 3,4-methoxyphenyl | 1·75 |

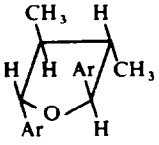
wish, but the proximity of the two desoxylignans (VI, VII) of the podophyllotoxin group to the conidendrins (VIII, IX) is probably due to the increased polarity of the trimethoxyphenyl group of the former being counteracted by the greater hindrance of their lactone carbonyl groups. The presence of an unhindered lactone group in acyclic lignans (Table 2) leads to a $\Delta t_r$ value of 1·2 between the dimethyl ethers of

TABLE 3. TETRAHYDROFURAN DERIVATIVES
($t_r$ RELATIVE TO CHOLESTANE IN PARENTHESIS)

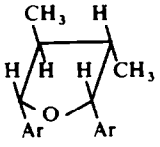
Series (a) has Ar = 3,4-dimethoxyphenyl
Series (b) has Ar = 3,4-methylenedioxyphenyl



XIIIa Galgravin (1·00)



XIVa Galbelgin (1·00)
XIVb Galbacin (0·55)



XVa Veraguensin (0·92)

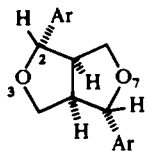
matairesinol (XII) and *meso*-dihydroguaiaretic acid (XI), similar to that (1·6) found by comparison of dimethyl-α-conidendrin (VIII) and galbulin (II), which are geometrically equivalent. Attention is drawn to the clear resolution of the conidendrin isomers (VIII, IX) and also to the separation of the retro-lactones (VI and VII).

Comparison of the group of monocyclic furans (Table 3) with analogous aryltetrahydronapthalenes (e.g. Table 1, X, Y = Me) shows that $t_r$ is extended in the former. This is to be expected in view of the inclusion of the more basic heterocyclic ether function and a slight increase in mol wt. However, the introduction of a second ether function in the tetrahydrofurans (Table 4) leads to an unexpectedly large further increment in $t_r$; this difference probably arises from a steric factor which causes a relative reduction of $t_r$ in the monocyclic group where *both* C2 and C5 are arylated. For the same reason resolution of geometrical isomers is less clear-cut here, galgravin (XIIIa) and galbelgin (XIVa) being incompletely resolved, although the effect of eclipsing one pair of aryl and Me substituents in veraguensin (XVa) is detectable. The bis-methylenedioxy compound galbacin (XIVb) shows a marked fall in $t_r$. Pelter *et al.* have obtained the mass spectra of a number of furanoid lignans[5,6] but were unable to detect any significant dependence on structure. In this context gas chromatography offers a useful advance, particularly when applied to bicyclic compounds (Table 4). The three diastereoisomers of the eudesmin group were resolved. Eudesmin (XVIa) itself with the most extended structure has the largest $t_r$ value (2·52); diaeudesmin (XVIIIa) with aryl substituents in 2β and 6β positions* eclipsing C8 and C4 respectively has the lowest value of 2·00, whilst epieudesmin (XVIIa) falls rationally in an intermediate position with $t_r$ 2·37. This sequence of results supplements the evidence of PMR spectra which was presented when diaeudesmin was first isolated.[7] The validity of this approach was confirmed when a similar elution sequence was determined for the three isomeric dimethyl lirioresinols (XVI → XVIIIc) here also the chromatogram affords additional evidence for the allocated structures.[8] The large $t_r$ values found for the dimethyl lirioresinols are further evidence of the importance of a polar factor. Our technique reached a limit here as mixtures of these compounds could not be resolved; nevertheless, lirioresinol A, with two free phenolic OH groups, was detected as a broad peak with $t_r$ 6·53.

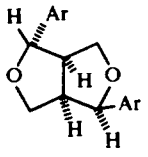
The presence of a tertiary OH group on more readily eluted parent structures does

* See Note on Nomenclature, page 4097.

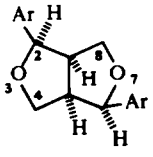
TABLE 4. TETRAHYDROFUROFURAN (DIOXACYCLO-OCTANE) DERIVATIVES SERIES (C) HAS Ar = 3,4,5-TRIMETHOXYPHENYL



XVI



XVII



XVIII

$t_r$ relative to cholestane in parenthesis

XVIa Eudesmin (2·52)

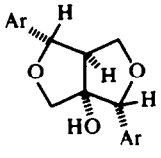
XVIIa Epieudesmin (2·37)
XVIIb Asarinin (1·74)

XVIIIa Diaeudesmin (2·00)

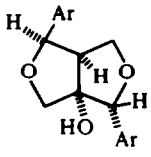
XVIc Dimethyl-lirioresinol B (5·47)

XVIIc Dimethyl-lirioresinol A (5·28)

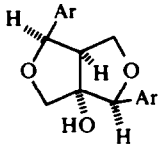
XVIIIc Dimethyl-lirioresinol C (5·12)



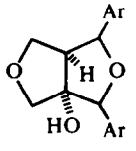
XIXa
Isogmelinol (3·01)



XXa
Gmelinol (2·74)



XXIa
Neogmelionol (2·89)



XXIIa

not lead to appreciable peak-broadening. Hydroxyotobain (IV, $t_r$ 0·52) was compared with the parent compound (0·39) and it was shown that the formation[9] of γ-dehydro-otobain[10] from it was complete within one minute. Although additional[11] evidence of the geometry at C1 of hydroxyotobain would be useful, the flexibility of ring B precludes any estimate of the relative hindrance of the OH group in the possible conformers. In contrast, the relative hindrance of an OH group on the almost rigid tetrahydrofurofuran structures can be evaluated and correlated with $t_r$ values for the gmelinols. Of this group, neogmelinol has been the subject of a recent structural revision[12] (XXIa for XXIIa). The similarity between its $t_r$ value and those of isomers of known[13] 2,6-diaryldioxacyclo-octanes is consistent with the revised structure (XXIa); a divergent value would be expected for a 2,4-diarylated compound (as XXIIa), as hindrance by two adjacent aryl groups markedly reduced the $t_r$ values of the tetrahydrofuran group (XIII–XV). The relative retention times of isogmelinol (XIXa, 3·02) neogmelinol (2·89), and gmelinol (XXa, 2·74) reveal a greater degree of skeletal compression in the latter pair. Neogmelinol and gmelinol differ only in the

relative configuration of the OH group and one aryl substituent, the former alcohol is less hindered and therefore has an increased retention time.

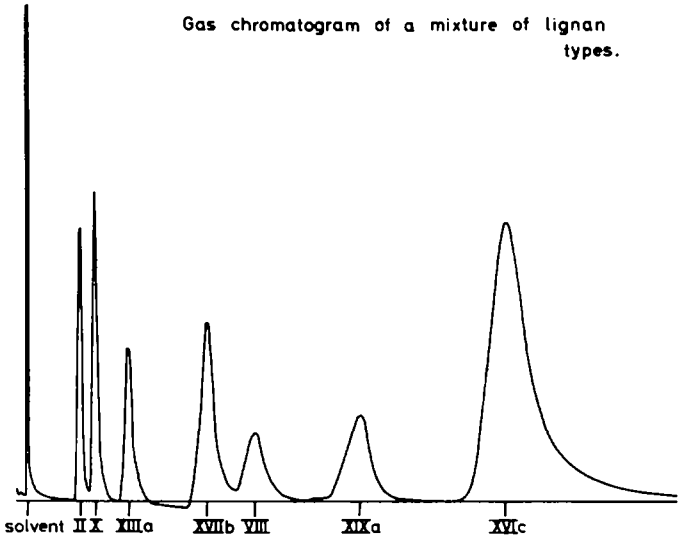



FIG. 1 Gas chromatogram of a mixture of lignan types.

not lead to appreciable peak-broadening. Hydroxytrobain (IV, $t_r$ 0·52) was compared

Reference to Fig. 1 indicates the relation between peak width, tailing, and retention time. It is evident that the procedure can be used to classify unknowns provided spectroscopic evidence of functionality is available: coupling to a mass spectrometer would give an effective combination.

*Note on Nomenclature*

We prefer to describe epimers in this way rather than to speak of "axial" and "equatorial" substitution on a *five-membered* ring. There need be no confusion with the older Greek symbols for position isomers provided the ring number is given and β substitution implies a *syn*-interaction with the adjacent ring-carbon atom. Earlier descriptions of a 2β-substituent as "axial" are inexact for models show (Fig. 2) that upward "flipping" of the ring oxygen partially relieves compression and in this conformation (A) the 2α-substituent is more nearly parallel to the axis of symmetry of the ring.

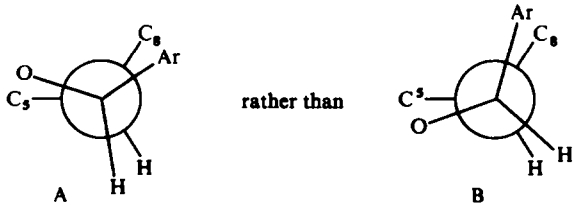



FIG. 2

## EXPERIMENTAL

*Chromatographic procedure.* A Hewlett-Packard, Facts and Methods model 402, instrument was used with a flame-ionization detector, which was coupled to a Hewlett–Packard recorder model 7127A. A 4 ft, U-shaped, glass column (4 mm i.d.) packed with UC-W98 (3·8%, equiv to SE-30) on celite (diatoport-S, 80–100 mesh) was employed at an efficiency of 440 theoretical plates/foot calculated[14] for cholestane.

Samples (1–2 μl) were injected directly on the column at 240° with no flash heating. The gas flow rates at room temp were: nitrogen—97 ml/min; hydrogen—43 ml/min; air—310 ml/min. The $N_2$ and $H_2$ flows were evaluated using a bubble flowmeter, but for this particular instrument the air flow was controlled by the rotameter setting. A Perkin–Elmer F11 instrument, purchased with a grant from the Royal Society Research Fund, also gave acceptable results. All quoted retention times were reproducible to within 1%.

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