Structural Analysis of the Urinary Lignan 2,3-Bis-(3-hydroxybenzyl)butan-4-olide ('Enterolactone'). A 400 MHz Nuclear Magnetic Resonance Study for the Solution State and *X*-Ray Study for the Crystal State

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Studies of the ^{13}C and ^{1}H (at 400 MHz) spectra of the title compound, with application of the nuclear Overhauser effect (n.O.e.) and decoupling difference methods, have permitted the first complete spectral assignments for a lignan of the dibenzylbutane type, including distinction between the two benzylic groups, which differ in their relationship to the lactone ring. It is now clear that previous analyses of n.m.r. spectra for some structurally related plant lignans have led to incorrect ^{1}H assignments, whereas ^{13}C assignments have previously been incomplete. An *X*-ray crystallographic study has established the conformation of enterolactone in the solid state, which corresponds to the preferred conformation in solution, inferred from n.m.r. data.

Phenolic fractions extracted from human or various animal urines have recently been found [1,2] to contain, in addition to the familiar steroidal oestrogens, significant quantities of novel phenolic compounds belonging to the structural class of dibenzylbutane lignans.[3]

Lignans of many types occur in plants, but had not previously been obtained from animal sources. The new lignans so far identified, (±)-*trans*-2,3-bis(3-hydroxybenzyl)butan-4-olide (1a),[1,2] for which we have proposed [4] the trivial name 'enterolactone,'† and (±)-2,3-bis(3-hydroxybenzyl)butane-1,4-diol (2),[2] 'enterodiol,'† differ from known plant lignans in lacking *para*-oxygen substitution in the aromatic rings. They are now known to originate in the gut [4-10] by microbial action on plant lignans of dietary origin. One such source has been identified [10] as a diglycoside (4) of 2,3-bis-(3-methoxy-4-hydroxybenzyl)butane-1,4-diol [secoisolariciresinol; (3)], which has been characterised from linseed and probably occurs in other plant products. A correlation of enterolactone excretion with vegetarian diet has been noted.[11] It is remarkable that the derived urinary lignans, unlike plant lignans,[3] are racemic.[12,13] The physiological significance of the new lignans is at present obscure; interest was initially aroused by the observation that their excretion by the female reaches a peak in the luteal phase of the menstrual cycle, and around weeks 14—20 of pregnancy, suggesting a controlling influence related to steroid hormone levels. The observation of enterohepatic circulation of these lignans,[9] the occurrence of enterolactone in seminal fluid at relatively high concentration,[14] and a reported effect on RNA synthesis,[15] all point to the need for further study.

The structures of the new lignans [1,2,16] were deduced from mass spectrometric, i.r., u.v., and n.m.r. data and were confirmed by chemical synthesis.[12,13] An improved synthesis of the dimethyl ether of compound (1) was reported recently.[17]

The symmetry of the butanediol lignan (2) results in simple ^{1}H and ^{13}C n.m.r. spectra [16] which were readily interpreted by inspection and by reference to published data for the related lignan dihydrocubebin (5). The lack of symmetry in enterolactone (1), however, leads to more complex spectra. The ^{1}H spectrum showed a striking resemblance in the high-field region to those of matairesinol (6a) and its dimethyl ether (6b). Previous publications,[18] our own [2] included, have contained the naive assumption that the signals appearing in the high-field part of the ^{1}H spectrum of a lignan of *trans*-2,3-dibenzylbutan-4-olide type (*e.g.*, matairesinol) could be assigned by inspection on the simple basis of integrated intensities and expected shifts. Thus the four-proton group at 2.5—2.6 p.p.m. was tentatively but incorrectly assigned [2] to the four benzylic protons (7- and 7'-positions) and the two-proton signal around 2.9 p.p.m. to the two methine protons [C(8) and C(8')] on the lactone ring.

We now report an analysis of the ^{1}H and ^{13}C n.m.r. spectra of enterolactone (1) which established that the two benzylic pairs of protons do not give superimposed resonances.‡ The two-proton signal near δ 2.9 arises from the benzylic methylene pair [C(7')] β to the lactone carbonyl group. The four-proton signal near δ 2.5—2.6 p.p.m. is a composite of resonances from the protons at C(7), C(8), and C(8'), which overlap in the 100 MHz spectrum. This study has afforded all the individual ^{1}H (Table 1) and ^{13}C chemical shifts (Table 4) including, we believe for the first time in such a compound, a full distinction between ^{13}C signals from the two similarly substituted aromatic rings. All the significant ^{1}H–^{1}H coupling constants are also reported (Table 2). The results for the aliphatic part of the molecule are directly applicable to matairesinol and similar compounds, and our procedures and conclusions should be of value in assigning the n.m.r. spectra of other related lignans.

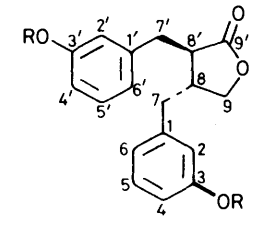
The conformational preference of enterolactone in solution, derived from n.O.e. data, was found to correspond with that in the solid state, obtained from an *X*-ray crystallographic study.

Results

Initial studies on enterolactone (1) were carried out at 100 MHz for protons and at 25.1 MHz for ^{13}C. The ^{1}H spectra showed a

† These compounds were originally designated, respectively, as HPMF (ref. 1) or HBBL (ref. 2) (now enterolactone), and HBBD (ref. 2) (enterodiol).

‡ We are grateful to Dr. M. B. Groen, Organon Scientific Development Group, Oss, Holland, for informing us that his group reached a similar conclusion from a study of the 250 MHz spectrum.



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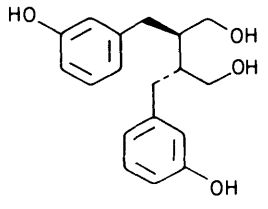
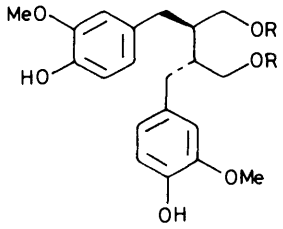
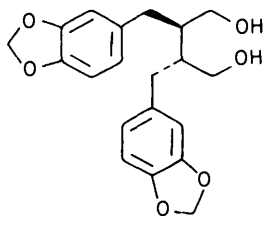
(1b) R = Me



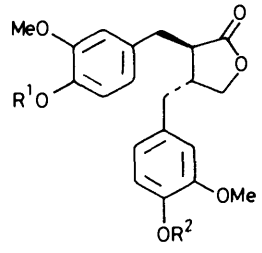
(2)



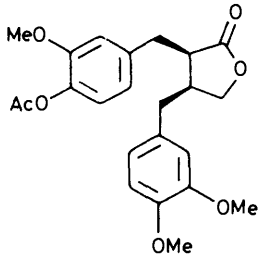
(3) R = H
(4) R = β-D-glycoside



(5)



(6a) $R^1 = R^2 = H$
(6b) $R^1 = R^2 = Me$
(7) R^1 = Ac, R^2 = Me



(8)

total of 18 protons, two of which were exchangeable with deuterium oxide. This agreed with mass spectral evidence [2,16] for a di-phenolic compound with the molecular formula $C_{18}H_{18}O_4$, the bis-trimethylsilyl derivative giving a molecular ion of *m/z* 442.

Proton Assignments.—At 100 MHz the ^1H n.m.r. spectrum was obviously complicated by heavy overlapping of signals and second-order coupling, which was also evident at 200 MHz. A number of chemical shifts and connectivities were established at these frequencies by homonuclear spin decoupling and spectrum simulation, but several features of the spectrum remained ambiguous. The greater dispersion of signals at 400 MHz permitted assignment of all chemical shifts and geminal, vicinal, *ortho* and *meta* coupling constants (Table 1) by a series of Fourier Transform Inter-Nuclear Double Resonance (FT INDOR), nuclear Overhauser effect difference spectra (NOEDS), partially relaxed 180°-τ-90° experiments, and solvent-shifted spectra. Spectral simulation permitted ultimate refinement of data.

A first appraisal of the spectrum (in $CDCl_3$, δ from internal Me_4Si) at 400 MHz (Figure 1) revealed two pairs of double doublets, the AB part of an ABM system at δ 2.91 and 3.00 and the AB part of an ABX system at δ 3.87 and 4.13. The integrals for each of the four signals were equal and were taken as one proton. There were also two heavily 'second-order coupled' multiplets at 2.5 and 2.6, each integrating for a further two protons, bringing the total number of aliphatic protons to eight. A broad resonance which varied in its chemical shift, both with concentration and with addition of different solvents [*e.g.* C_5D_5N or $(CD_3)_2CO$] to the deuteriochloroform, and exchanged with deuterium on addition of D_2O, was assigned to two phenolic hydroxy protons. Four groups of complex signals at low field (Figure 2), which resolved into a fine triplet at δ 6.47 integrating for one proton, a multiplet centred at δ 6.6 for two protons, a multiplet centred at δ 6.72 for three protons, and two mutually non-coupled overlapping triplets each integrating for one proton at 7.14 and 7.18, were assigned to a total of eight aromatic protons. Thus the spectrum integrated for 18 protons altogether, agreeing well with the mass spectral evidence.

Table 1. Proton chemical shifts for enterolactone (1a) and its dimethyl ether (1b): data obtained at 400 MHz, 300 K

	δ (±0.002) [a] from internal Me_4Si [b]					
Proton	1	2	3	4	5	Multiplicity
2	6.468	6.552	6.473	6.580	6.55	t
2′	6.594	6.720	6.608	6.738	6.75	t
3-OH	4.930	9.9	5.06	7.9	3.69 (OMe)	bs
3′-OH	4.930	9.9	5.06	7.9	3.70 (OMe)	bs
4	6.700	6.737	6.705	6.725	6.77	m
4′	6.738	6.762	6.736	6.760	6.79	m
5	7.145	7.095	7.142	7.090	7.19	t
5′	7.177	7.123	7.175	7.122	7.23	t
6	6.602	6.529	6.583	6.530	6.61	dt
6′	6.726	6.687	6.728	6.695	6.78	dt
7_A(*pro-R* [c])	2.500	2.432	2.499	2.415	2.49	m
7_B(*pro-S* [c])	2.610	2.620	2.611	2.610	2.63	m
$7'_A$(*pro-S* [c])	2.906	2.885	2.905	2.867	2.92	dd
$7'_B$(*pro-R* [c])	3.000	3.025	3.002	2.985	3.06	dd
8	2.498	2.555	2.497	2.530	2.53	m
8′	2.590	2.585	2.589	2.585	2.62	m
9_A(*pro-R* [c])	3.868	3.848	3.869	3.828	3.86	dd
9_B(*pro-S* [c])	4.135	4.096	4.133	4.049	4.11	dd

[a] As refined by spectrum simulation. [b] 1, Natural lignan, 1.2 mg in 0.5 ml $CDCl_3$; 2, natural lignan, 0.9 mg in 0.45 ml $CDCl_3$ + 0.008 ml C_5D_5N; 3, synthetic lignan, 6.2 mg in 0.35 ml $CDCl_3$; 4, synthetic lignan, 75 mg in 0.3 ml $CDCl_3$ + 0.2 ml $(CD_3)_2CO$; 5, lignan dimethyl ether, 42 mg in 0.55 ml $CDCl_3$. [c] With reference to the (8*R*,8′*R*) enantiomer (see Figure 8).

*Aliphatic protons.** The chemical shifts of the AB pair of signals of the ABX system (δ 3.87 and 4.13) identified them as originating from $H(9_A)$ and $H(9_B)$, on carbon adjacent to oxygen. Homonuclear FT INDOR [Figure 3, (A) and (B)], observed in the difference mode, unequivocally located the signal from the X proton of this ABX system [H(8)] in the two-proton multiplet at δ 2.5. Partially relaxed FT spectra using a 180°-τ-90° sequence, with τ values of 0.37 s and 0.56 s to null methylene and methine proton signals, respectively (Figure 4), confirmed that X [H(8)] was a methine proton and revealed the other methine proton [H(8′)] signal under the δ 2.6 multiplet. In the partially relaxed spectrum with τ = 0.37 s, to null the signals from geminal benzylic methylene protons, the signal from the M proton [H(8′)] appeared, phase inverted, as a double triplet at δ 2.59. The X proton, heavily second-order coupled, still appeared as a complex multiplet.

* For convenient reference in discussing n.m.r. signals from individual protons in methylene groups [at C(7), (7′), and (9)], the protons within each pair are distinguished by subscripts A or B (for the protons giving the higher- and lower-field signals, respectively). See also the Discussion section.

Table 2. ^1H–^1H Coupling constants [a] for enterolactone or its dimethyl ether (Hz ± 0.05)

Aromatic protons	*ortho* ^3J	*meta* ^4J	Aliphatic protons	^2J	^3J
2,4		1.9	$7_A,7_B$	13.0	
2′,4′		1.9	$7'_A,7'_B$	13.8	
2,6		1.2	$7_A,8$		8.6
2′,6′		1.2	$7_B,8$		5.4
4,5	7.9		$7'_A,8'$		7.1
4′,5′	7.9		$7'_B,8'$		5.1
4,6		0.9	$8,8'$		7.8
4′,6′		0.9	$8,9_A$		7.6
5,6	7.8		$8,9_B$		7.0
5′,6′	7.8		$9_A,9_B$	9.1	

[a] All coupling constants obtained by simulation, LAOCN III (ref. 19).

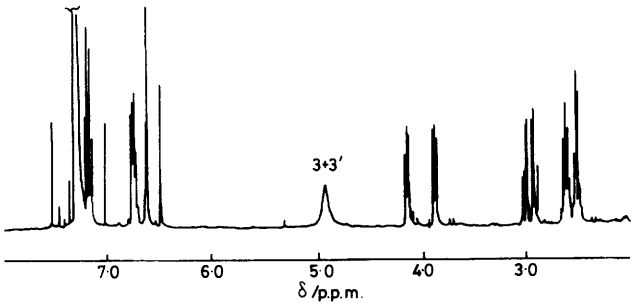


Figure 1. 400 MHz spectrum of natural enterolactone (1a) (1.2 mg in 0.5 ml $CDCl_3$)

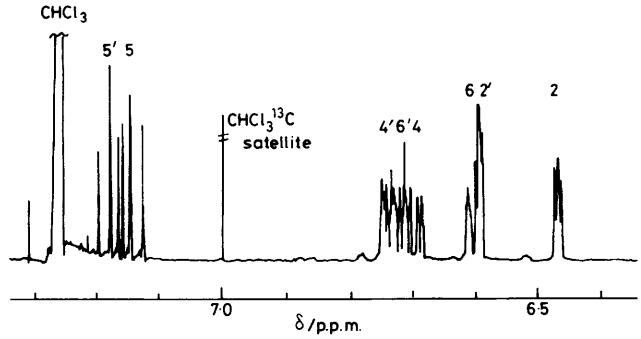


Figure 2. Aromatic proton signals of enterolactone: expanded scale (400 MHz)

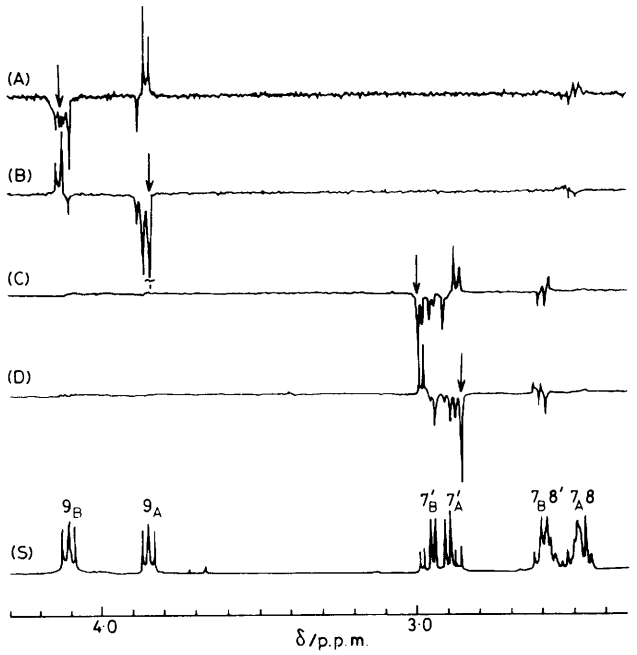


Figure 3. Homonuclear FT INDOR by difference: → indicates transitions irradiated. (S) Standard expansion of aliphatic protons; (A) irradiation of left central transition of proton 9_B; (B) irradiation of highest-field transition of proton 9_A; (C) irradiation of lowest-field transition of proton $7'_B$; (D) irradiation of highest-field transition of proton $7'_A$

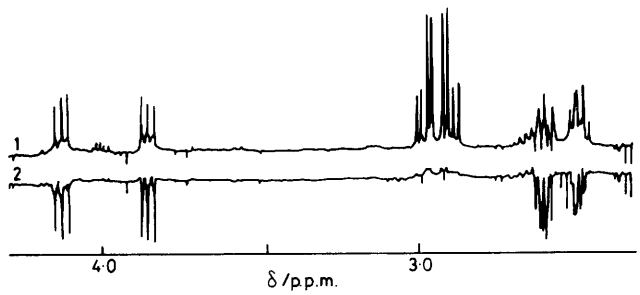


Figure 4. Partially relaxed 180-τ-90 spectra of aliphatic protons. (1) Methylene protons appearing when τ = 0.56 s, methines nulled; (2) methine protons appearing when τ = 0.37 s

Another FT INDOR experiment [Figure 3, (C) and (D)] on the AB protons of the ABM system, at δ 2.91 and 3.00, confirmed the assignment of M as the methine proton [H(8′)] in the two-proton multiplet at *ca.* δ 2.6. The coupled signals at δ 2.91 and 3.00 therefore resulted from the benzylic pair [$H(7'_A)$ and $(7'_B)$] closest to the carbonyl group of the lactone ring. The remaining two protons, the benzylic pair $H(7_A)$ and $H(7_B)$, correspond to those parts of the multiplets at *ca.* δ 2.5 and 2.6, respectively, not accounted for by the overlapping signals from the two methine protons.

Small additions of polar solvents such as deuteriopyridine or deuterioacetone to the solutions in deuteriochloroform resulted in small shifts of the high-field group of signals relative to each other. As a consequence some of the second-order complexity was removed. The highest field signal [$H(7_A)$], for example, shifted from δ 2.50 to 2.42 on addition of deuterioacetone, and was then clearly a double doublet coupled to the X proton [H(8)]; the latter had shifted downfield from δ 2.50 to 2.53.

This analysis established the main n.m.r. features of the central aliphatic part of the molecule. The *trans*-configuration of the two benzylic groups had previously been inferred [16] from spectral comparisons with some other compounds of dibenzylbutane type, including matairesinol (6a) and dihydrocubebin (5). We note also a close resemblance of the high-field part of the spectrum of enterolactone to that reported [18] for arctigenin acetate (7), of *trans*-configuration, rather than to that of the *cis*-isomer, isoarctigenin (8), although in the light of our present findings the ^1H assignments for these two compounds (7) and (8) were not wholly accurate. The *trans*-configuration of enterolactone was confirmed in the present work by *X*-ray crystallographic analysis and by nuclear Overhauser effects (see later) in the ^1H n.m.r. spectrum.

Table 3. Nuclear Overhauser effects observed by difference [a]

	Proton irradiated	Large +ve (15—40%)	Medium +ve (5—15%)	Small +ve (0.5—5%)	Small −ve (0.5—5%)
Enterolactone [b]	$7'_A$	$7'_A$		8	8′
	$7'_B$	$7'_B$	8′		
	9_A	9_A		$7_A,7_B,8$	
	9_B	9_B	8		$7_A,7_B$
	2 (partial 6)			$7_B,7_A,8,9_A$	
	2′,4′,4,6′			$7'_B,7'_A,7_B,8$	
Dimethyl ether [c]	$7'_A$	$7'_B$	2′,6′	8,2,6	8′
	$7'_B$	$7'_A$	8′,2′,6′		
	9_A (partial 3,3′)	9_B	2	$7_B,7_A,6,4$	
	9_B	9_A	8	2,4	3
	2 (partial 6)		3	$7_A,7_B,8,9_A,5$	4
	6 (partial 2)	5	3,3′	$7_A,7_B,8$	4,2′,4′

[a] Samples were not degassed and hence results are simply qualitative. Aromatic protons were observed both as positive and negative n.O.e.s in all spectra owing to the rapid exchange of phenolic protons 'scrambling' other n.O.e.s. [b] Synthetic enterolactone [75 mg in 0.3 ml $CDCl_3$ + 0.2 ml $(CD_3)_2CO$ at 300 K]. [c] Dimethyl ether of enterolactone (42 mg in 0.55 ml $CDCl_3$) at 300 K.

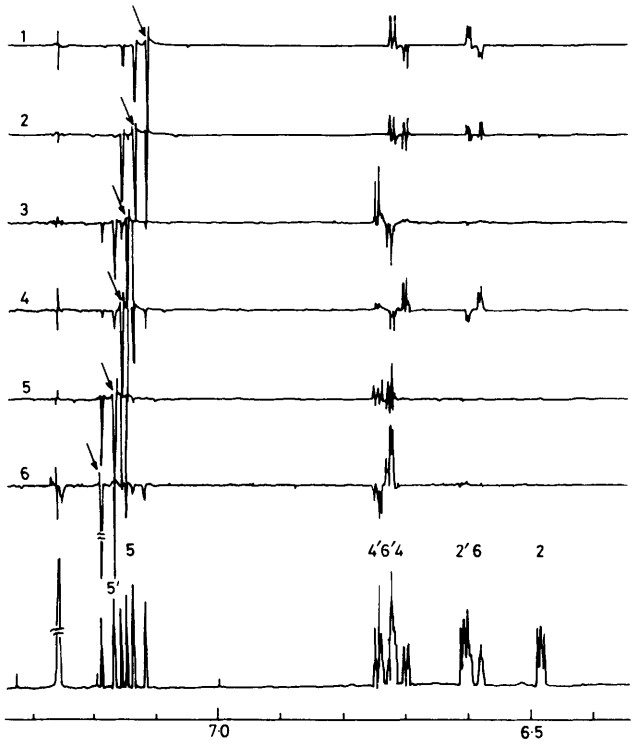


Figure 5. Homonuclear FT INDOR by difference: → indicates irradiation of H(5) and H(5′) transitions (6.2 mg in 0.35 ml $CDCl_3$)

Aromatic protons. By combining data obtained at 100, 200, and 400 MHz it was established that the lowest field signals, at δ 7.14 and 7.18, were two overlapping triplets, each exhibiting *J* values of 7.8 Hz, typical of *ortho* couplings. A series of FT INDOR difference experiments (Figure 5) on the six transitions giving rise to these triplets demonstrated their connectivities, each to two other signals among the higher field aromatic protons. The signal at δ 7.14 was *ortho* coupled to the double triplet at δ 6.58, and to the overlapping signal centred at δ 6.71. Likewise the remaining triplet at δ 7.18 was *ortho* coupled to two signals at δ 6.73 and 6.74.

Relatively high-field chemical shifts for some of the aromatic protons (6.47—6.75 p.p.m. in deuteriochloroform solution) were indicative of hydroxy substitution.[20,21] The highest field signal at δ 6.47 appeared as a fine coupled triplet, with *J* values of 1.9 and 1.2 Hz (resolution enhancement revealed a double doublet). This, and a very similar signal at δ 6.60, overlapped by the double triplet at δ 6.58, indicated two protons with only *meta* couplings. These observations left only the conclusion that there were two *meta*-substituted phenolic rings, in non-identical local magnetic environments.

To complete the assignments of proton signals it was deemed desirable to use, to the full, the advantages of the computer-controlled 400 MHz spectrometer to achieve an unambiguous distinction between the pairs of protons within each of the three pro-chiral methylene groups, and to establish beyond doubt which aromatic ring should be assigned to each coupled set of aromatic protons. These remaining problems were solved by using ^1H–^1H n.O.e. difference measurements.

Nuclear Overhauser Effect (n.O.e.) Difference Measurements.—Homonuclear n.O.e., a manifestation of the mutual relaxation of nuclei, is inversely proportional to the sixth power of the inter-proton distance.[22–25] Thus in contrast to *J*, the scalar coupling constant which provides information about nuclei connected through 2—5 bonds, the n.O.e. provides information relating to spatial proximity of magnetic nuclei. Although only qualitative, the results (Table 3) serve to remove the remaining ambiguities mentioned above. Owing to rapid proton exchange between phenolic hydroxy protons and traces of water in the solvent, all n.O.e. measurements carried out on enterolactone itself showed appreciable polarization transfer to the hydroxy resonances, with a consequent scrambling of results particularly amongst the aromatic protons. The dimethyl ether (1b) of enterolactone was therefore also studied, yielding significantly better results. The ^1H and ^{13}C spectra of enterolactone dimethyl ether (Tables 1 and 4) are so similar in detail to those of enterolactone itself as to justify the use of the derivative as a model for n.O.e. work. Moreover, those features of the n.O.e.s which could be observed for free enterolactone without interference by traces of water left no doubt as to the conformational similarity of the two molecules.

We therefore describe the n.O.e. experiments on enterolactone dimethyl ether (1b) in detail, and summarize the very similar findings for enterolactone itself (Table 3).

Selective irradiation of the lowest field aliphatic resonance at δ 4.10 [$H(9_B)$] in $CDCl_3$ [Figure 6(a)], by a weak decoupling r.f. field gated off during acquisition, resulted in a large positive enhancement of the signal from its geminal partner at δ 3.86

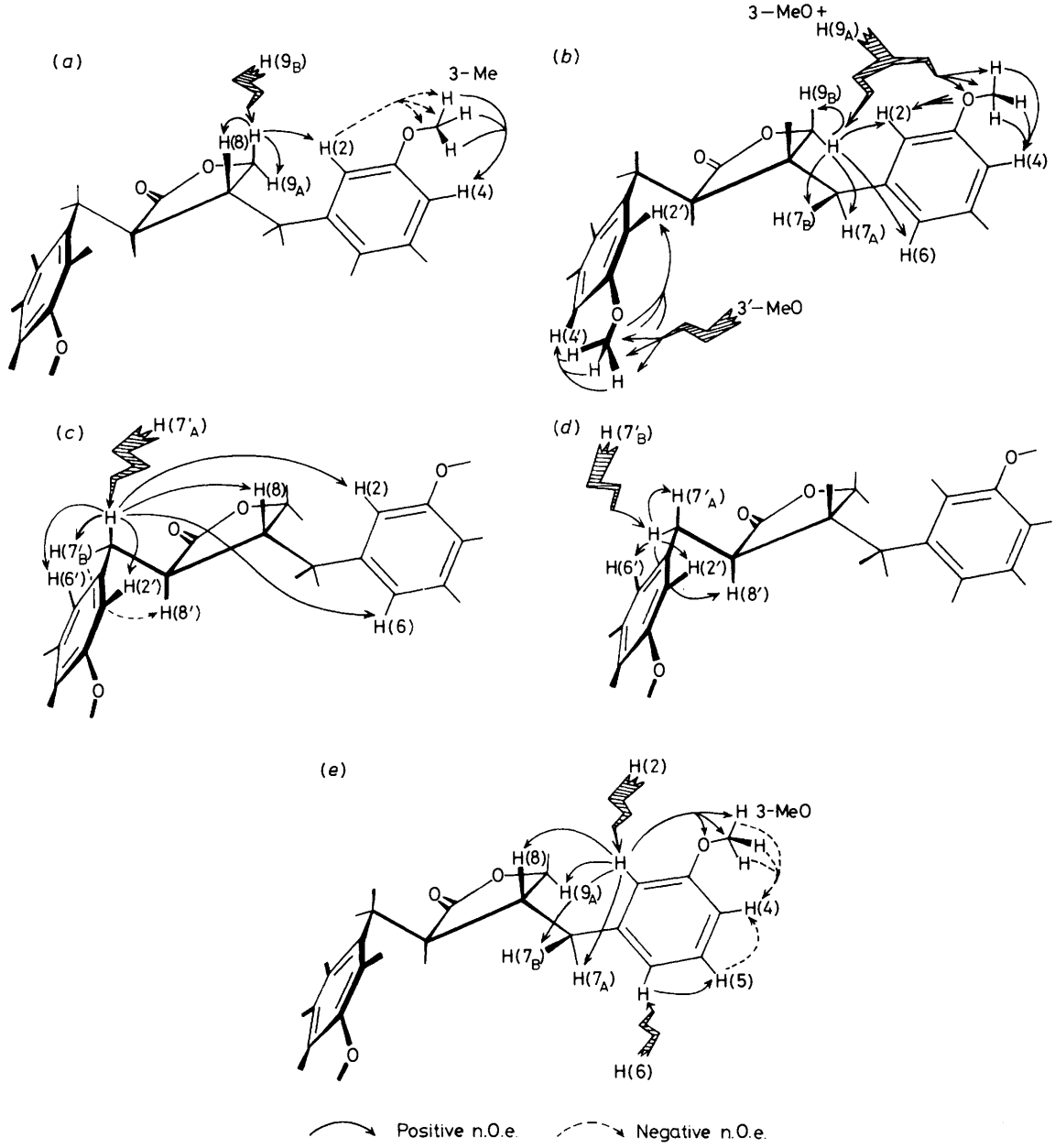


Figure 6. Nuclear Overhauser enhancements in enterolactone dimethyl ether (observed by difference). Irradiation of: (*a*) $H(9_B)$; (*b*) $H(9_A)$; (*c*) $H(7'_A)$; (*d*) $H(7'_B)$; and (*e*) H(2) and H(6)

$[H(9_A)]$, accompanied by a smaller positive enhancement of the resonance at δ 2.53 [H(8)], a large negative n.O.e. on the higher field methoxy singlet at δ 3.69 and very small positive enhancements of the fine triplet at δ 6.55 [H(2)] and the double triplet at δ 6.77 [H(4)]. The corroborating experiment was the irradiation at the frequency of the signal at δ 3.86 $[H(9_A)]$ [Figure 6(b)], which resulted in a large positive enhancement for $H(9_B)$ and much smaller positive n.O.e.s for $H(7_A)$, $H(7_B)$ and the aromatic protons H(2), H(4), and H(6). The two methoxy resonances, being close in frequency terms to the irradiation, were partially saturated resulting in a further complication of the observed aromatic n.O.e.s [Figure 8(b)]. However, the two results together specify $H(9_B)$ as lying *cis* to H(8) and $H(9_A)$ as *trans* to H(8) but close to the higher-field benzylic methylene pair $H(7_A)$ and $H(7_B)$. Furthermore the n.O.e.s between protons at C(9) and those on the nearest aromatic ring, including the higher-field methoxy group, gave the first clear means of distinguishing between the two sets of aromatic resonances.

Confirmatory results came from irradiation of the signal at 2.92 $[H(7'_A)]$[Figure 6(c)], which caused a very large positive enhancement of its geminal partner $[H(7'_B)]$ at δ 3.06 p.p.m., and a smaller positive enhancement of the signals from the two nearest aromatic protons, appearing as a fine triplet at δ 6.75 [H(2')] and a double triplet at δ 6.78 [H(6')]. There was also a smaller positive enhancement for H(8) at δ 2.53, and a weak *negative* n.O.e. for the resonance at δ 2.63 [H(8')]. The complementing experiment, wherein the geminal partner resonating at δ 3.06 p.p.m. $[H(7'_B)]$ [Figure 6(d)] was irradiated, gave a large positive enhancement for $H(7'_A)$ and a smaller positive n.O.e. for the H(8') signal at δ 2.62, together with a small positive n.O.e. on the two aromatic resonances arising from H(2') and H(6'). Thus the two lower-field benzylic methylene resonances $[H(7'_A)$ and $H(7'_B)]$ arise from protons adjacent to the

Table 4. ^{13}C Chemical shifts [a] for enterolactone (1a) and its dimethyl ether (1b)

Carbon	Sample [b] 1	2	3	4
1	139.3	139.5	139.3	139.2
1′	138.8	139.0	138.9	139.0
2	115.7	115.5	115.2	114.2
2′	116.2	116.2	115.7	114.6
3	157.6	156.0	156.6	159.4 (54.9) [c]
3′	157.6	156.1	156.7	159.4 (54.9) [c]
4	113.7	113.8	113.2	111.6
4′	114.0	114.0	113.4	112.0
5	129.3	129.8	129.1	129.28
5′	129.3	129.8	129.1	129.35
6	119.2	120.6	119.5	120.6
6′	119.9	121.3	120.2	121.3
7	38.3	38.3	37.7	38.3
7′	34.9	34.9	34.3	34.9
8	41.0	41.1	40.7	41.1
8′	46.1	46.2	45.7	46.1
9	71.0	71.4	70.6	70.9
9′	178.5	178.9	178.1	178.0

[a] Measured from Me_4Si as internal standard at 300 K. [b] 1, Natural enterolactone, 0.4 mg in 20 μl $CDCl_3$ + 10 μl C_5D_5N; 25.1 MHz; 300 K; 2, synthetic enterolactone, 6.2 mg in 0.35 ml $CDCl_3$; 25.1 MHz; 300 K; 3, Synthetic enterolactone, 75 mg in 0.3 ml $CDCl_3$ + 0.2 ml $(CD_3)_2CO$; 100 MHz; 300 K; 4, dimethyl ether of enterolactone, 42 mg in 0.55 ml $CDCl_3$; 67.8 and 25.1 MHz; 300 K. [c] Methoxy carbons.

aromatic ring designated by primed locants. All the protons associated with the benzylic group on the carbonyl side of the lactone ring therefore resonate at lower field than those on the other benzylic group.

The negative n.O.e.[23,25,26] between $H(7'_A)$ and H(8′), contrasting with the positive n.O.e. between $H(7'_B)$ and H(8′), is interpreted as a three-spin effect through $H(7'_B)$, requiring that the preferred rotamer about the C(7′)–C(8′) bond is one with $H(7'_B)$ *gauche* (or synclinal) and $H(7'_A)$ antiperiplanar to H(8′). This conclusion is supported by the smaller vicinal coupling constant $J_{7'_B,8'}$ (5.1 Hz) in contrast to the $J_{7'_A,8'}$ (7.1 Hz). The preferred conformation about the 7′–8′ bond deduced for the compound in solution corresponds to that found in the crystal (see later). The small positive n.O.e. between $H(7'_A)$ and H(8) is also compatible with this conformation, both protons being on the same face of the lactone ring, as required by the *trans*-disubstitution.

As a further test of assignments of individual sets of aromatic resonances, n.O.e.s were observed while simultaneously irradiating the aromatic protons H(2) (δ 6.55) and H(6) (δ 6.61). Apart from n.O.e.s for the other protons of the same (un-primed) ring, positive n.O.e.s were observed for $H(7_A)$, $H(7_B)$, H(8), and $H(9_A)$ [Figure 6(e)], again compatible with the conformation illustrated in the Figures.

All the ^1H chemical shifts and ^1H–^1H coupling constants were ultimately refined by spectral simulation, using an eight-spin modified LAOCN III program.[19] Indeed this was the only way of obtaining the $^3J_{8,8'}$ coupling of 7.8 Hz accurately. It is interesting to note that the aromatic protons showed no measurable *para* couplings, or extra-ring couplings to the benzylic methylene protons.

^{13}C Assignments.—Having obtained a satisfactory data set for the proton spectrum a full analysis of the ^{13}C spectrum was possible.

For enterolactone (1a) the ^{13}C spectrum at 25.1 MHz contained a total of 17 distinguishable carbon resonances (Table 4). (Chemical shifts mentioned in the text are those in column 2 or 3 of Table 4.) The lowest field signal at δ 178.9 was obviously from the lactone carbonyl carbon atom. The region to high field of the solvent deuteriochloroform triplet showed five non-aromatic carbon resonances. The off-resonance proton-decoupled spectrum for this region revealed two triplets at highest field, then two doublets and a further triplet, in accordance with the total of eight aliphatic protons. Eleven resolvable aromatic carbon resonances were observed. The off-resonance decoupled spectrum distinguished four non-protonated from seven protonated carbons. Integration of a non-nuclear Overhauser enhanced broad-band proton-decoupled spectrum, with a suitable relaxation delay between pulses, clearly showed the resonance at 129.8 p.p.m. to derive from two carbons, confirming the total of 18.

The C(9) carbon was trivially assignable to the δ 71.4 resonance (adjacent to oxygen). Selective proton decoupling established the origins of the other high-field ^{13}C signals. Thus irradiation of $H(7'_A)$ and $H(7'_B)$ (δ 2.95) caused the carbon resonance at δ 34.9 [C(7′)] to experience almost complete decoupling. Similarly irradiation of the protons resonating at δ 2.5 [$H(7_A)$ and H(9)] resulted in a partial decoupling at δ 38.3 [C(7)] and near complete decoupling at δ 41.1 [C(8)]. The same effect was observed in the ^{13}C spectrum at δ 38.3 on selectively decoupling $H(7_B)$ and H(8′) (δ 2.6) with a corresponding nearly complete decoupling of the resonance at δ 46.2, hence assigned to C(8′).

Among the aromatic carbons, only the C(3) and C(3′) pair of signals at δ 156.0 and 156.1, and the C(1) and C(1′) pair at δ 139.5 could be assigned with confidence on the basis of their shifts, although distinction within each pair was not possible at this point (however, see later). Selective irradiation of the H(5) and H(5′) protons (triplets, *ca.* δ 7.17) decoupled the two-carbon resonance at δ 129.8, confirming it as corresponding to C(5) and C(5′).

The experiments described were carried out for protons at 100 MHz and observation of ^{13}C at 25.1 MHz, but it was necessary to use the greater proton dispersion at 400 MHz to achieve further ^{13}C assignments. By observing ^{13}C at 100 MHz and making use of the knowledge gained from 400 MHz ^1H spectra concerning proton resonances from each aromatic ring, we were able to construct a ^1H–^{13}C correlation plot of data from a stepped-frequency series of ^{13}C-{^1H} experiments,[27] which allowed discrimination between the resonances due to protonated carbon arising from each of the aromatic rings.

Discrimination between the resonances from the non-protonated carbon atoms in the aromatic rings was more difficult. Two experiments were carried out to distinguish C(1) and C(1′). The first involved irradiating the benzylic methylene proton pair $H(7'_A)$ and $H(7'_B)$ at δ 2.95. A careful comparison of the resulting ^{13}C spectrum and the completely coupled non-Overhauser enhanced spectrum revealed that the higher field of the two ^{13}C resonances, at 139.0 p.p.m., experienced a removal of some of the multiple fine couplings, together with an enhancement. Irradiation of the high-field protons H(2) and H(6), which both resonate close to δ 6.55, resulted in the lower-field ^{13}C resonance at δ 139.5 experiencing a marked perturbation as compared with the higher-field signal. The two results together assign C(1) to the resonance at δ 139.5 and C(1′) to the signal at δ 139.0.

A similar selective decoupling of the group of protons around δ 6.76 [H(2′), (4′), (6′) and (4)] resulted in a marked perturbation of both the ^{13}C resonances at δ 156.1 and 139.0 compared with those of their immediate partners, in addition to the expected decoupling of the resonances from the attached carbons at δ 115.5, 114,0, 121.3, and 113.8. Hence C(3′)

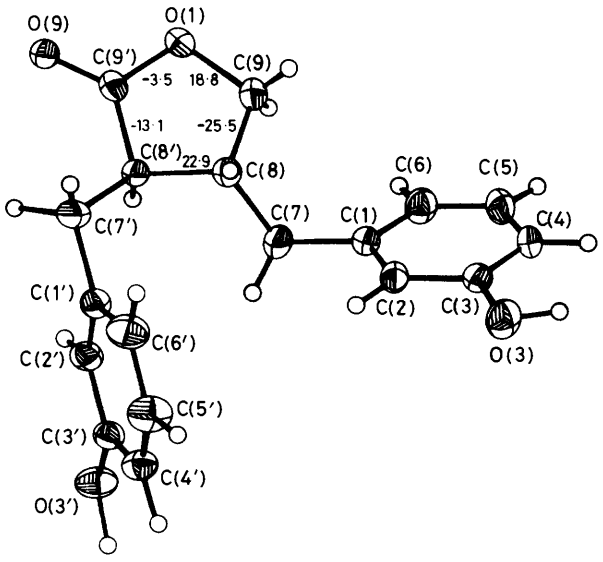


Figure 7. *X*-Ray crystallographic structure of enterolactone [showing a molecule of (8*R*,8′*R*) configuration] and principal ring torsion angles

Table 5. Bond lengths (Å) and bond angles (°) for enterolactone (*cf.* Figure 7)

Bond	Length	Bond	Length
O(1)–C(9)	1.461(2)	O(1)–C(9′)	1.331(2)
C(9′)–O(9)	1.214(2)		
C(9)–C(8)	1.526(2)	C(9′)–C(8′)	1.500(2)
C(8)–C(7)	1.522(2)	C(8′)–C(7′)	1.519(2)
C(7)–C(1)	1.505(2)	C(7′)–C(1′)	1.507(2)
C(1)–C(2)	1.383(2)	C(1′)–C(2′)	1.380(2)
C(2)–C(3)	1.388(2)	C(2′)–C(3′)	1.383(2)
C(3)–O(3)	1.367(2)	C(3′)–O(3′)	1.379(2)
C(3)–C(4)	1.388(2)	C(3′)–C(4′)	1.371(2)
C(4)–C(5)	1.384(2)	C(4′)–C(5′)	1.368(2)
C(5)–C(6)	1.372(2)	C(5′)–C(6′)	1.385(2)
C(1)–C(6)	1.382(2)	C(1′)–C(6′)	1.385(2)

Bonds	Bond angle	Bonds	Bond angle
C(9)–O(1)–C(9′)	109.9(1)	O(1)–C(9′)–C(8′)	111.6(1)
O(1)–C(9)–C(8)	105.8(1)	C(8′)–C(9′)–O(9)	129.0(1)
O(1)–C(9′)–O(9)	119.4(1)	C(9′)–C(8′)–C(8)	103.6(1)
C(9)–C(8)–C(8′)	102.4(1)	C(9′)–C(8′)–C(7′)	112.2(1)
C(9)–C(8)–C(7)	112.3(1)	C(7′)–C(8′)–C(8)	117.7(1)
C(7)–C(8)–C(8′)	115.2(1)	C(8′)–C(7′)–C(1′)	112.9(1)
C(8)–C(7)–C(1)	111.4(1)	C(7′)–C(1′)–C(2′)	120.8(1)
C(7)–C(1)–C(2)	120.4(1)	C(7′)–C(1′)–C(6′)	120.6(1)
C(7)–C(1)–C(6)	121.6(1)	C(2′)–C(1′)–C(6′)	118.6(1)
C(2)–C(1)–C(6)	118.1(1)	C(1′)–C(2′)–C(3′)	120.4(1)
C(1)–C(2)–C(3)	121.0(1)	C(2′)–C(3′)–C(4′)	120.8(1)
C(2)–C(3)–C(4)	120.2(1)	C(2′)–C(3′)–O(3′)	117.4(1)
C(2)–C(3)–O(3)	118.1(1)	C(4′)–C(3′)–O(3′)	121.7(1)
C(4)–C(3)–O(3)	121.7(1)	C(3′)–C(4′)–C(5′)	119.2(1)
C(3)–C(4)–C(5)	118.9(1)	C(4′)–C(5′)–C(6′)	120.6(1)
C(4)–C(5)–C(6)	120.3(1)	C(5′)–C(6′)–C(1′)	120.4(1)
C(5)–C(6)–C(1)	121.6(1)		

was assigned to the δ 156.1 p.p.m. signal and C(3) to the resonance at δ 156.0.

X-Ray Results.—In order to compare the state of the molecule in solution with that in the crystal an *X*-ray crystallographic study was carried out on synthetic racemic enterolactone.

Table 6. Fractional atomic co-ordinates and equivalent isotropic thermal parameters [a] for enterolactone

Atom	*x*	*y*	*z*	*B*(Å²)
O(1)	0.730 5(1)	0.527 8(2)	0.437 2(2)	5.30(4)
C(1)	0.570 9(1)	0.171 6(2)	0.526 1(2)	3.54(4)
C(2)	0.588 3(1)	0.106 7(2)	0.639 5(2)	3.71(5)
C(3)	0.515 2(2)	0.935 5(2)	0.698 2(2)	3.60(5)
O(3)	0.536 2(1)	0.024 6(2)	0.808 2(1)	5.17(4)
C(4)	0.423 4(2)	0.147 6(2)	0.644 4(2)	4.12(5)
C(5)	0.406 1(2)	0.213 3(3)	0.531 2(2)	4.80(6)
C(6)	0.479 2(2)	0.225 2(3)	0.474 1(2)	4.64(5)
C(7)	0.650 3(2)	0.182 9(2)	0.462 4(2)	4.07(5)
C(8)	0.715 5(2)	0.304 5(2)	0.500 0(2)	3.67(4)
C(9)	0.659 6(2)	0.434 0(3)	0.460 3(3)	5.83(6)
C(1′)	0.888 7(1)	0.105 9(2)	0.527 4(2)	3.54(4)
C(2′)	0.872 8(1)	0.017 3(2)	0.428 9(2)	3.45(4)
C(3′)	0.865 0(2)	−0.116 9(2)	0.448 6(2)	3.44(4)
O(3′)	0.851 0(1)	−0.199 8(2)	0.346 6(1)	4.91(4)
C(4′)	0.872 9(2)	−0.165 4(2)	0.565 8(2)	4.03(5)
C(5′)	0.889 1(2)	−0.078 8(3)	0.664 1(2)	5.07(6)
C(6′)	0.896 4(2)	0.056 4(3)	0.645 6(2)	4.82(6)
C(7′)	0.896 1(2)	0.253 0(2)	0.506 7(2)	4.09(5)
C(8′)	0.798 4(1)	0.315 6(2)	0.439 0(2)	3.24(4)
C(9′)	0.806 9(1)	0.462 3(2)	0.420 8(2)	3.53(4)
O(9′)	0.870 4(1)	0.523 2(2)	0.391 5(1)	4.13(3)
H(2)	0.154(2)	0.430(2)	0.183(2)	4.9(6)
H(4)	0.868(2)	0.362(2)	0.193(2)	6.0(6)
H(5)	0.837(2)	0.245(2)	−0.011(2)	5.6(6)
H(6)	0.468(2)	0.260(3)	0.396(2)	6.7(7)
H(7A)	0.617(2)	0.183(2)	0.367(2)	5.7(6)
H(7B)	0.191(2)	0.395(2)	−0.013(2)	4.8(5)
H(8)	0.245(1)	0.199(2)	0.087(2)	3.5(5)
H(9A)	0.366(2)	0.519(2)	0.477(2)	5.6(6)
H(9B)	0.604(2)	0.427(3)	0.373(3)	10.0(9)
H(2′)	0.867(1)	0.047(2)	0.343(2)	4.5(5)
H(4′)	0.135(2)	0.249(2)	0.422(2)	5.0(6)
H(5′)	0.105(2)	0.112(2)	0.253(2)	5.9(6)
H(6′)	0.402(2)	0.381(3)	0.221(2)	6.8(7)
H(7A′)	0.945(2)	0.274(2)	0.466(2)	4.8(5)
H(7B′)	0.417(2)	0.199(2)	0.076(2)	5.6(6)
H(8′)	0.774(2)	0.283(2)	0.347(2)	4.4(5)
H[O(3)]	0.520(2)	0.998(3)	0.166(3)	11.1(9)
H[O(3′)]	0.650(2)	0.205(3)	0.131(2)	10.6(7)

[a] The equivalent isotropic thermal parameter for C and O atoms is defined as:

$$\tfrac{1}{3}[a^2 . \beta(1,1) + b^2 . \beta(2,2) + c^2 . \beta(3,3) + ab\cos\gamma . \beta(1,2) + ac\cos\beta . \beta(1,3) + bc\cos\gamma . \beta(2,3)]$$

Values for hydrogen atoms are experimentally determined values.

A diagram of one molecule, with the relevant atom-numbering scheme, is shown in Figure 7. Bond lengths and angles are given in Table 5, and atomic co-ordinates in Table 6. Although the diagram shows a molecule with the (8*R*, 8′*R*)-configuration, it must be noted that the crystals are racemic and contain equal numbers of the (*R*,*R*) and (*S*,*S*) forms. The conformation observed for each molecule in the crystal corresponds to that which is believed to be most populated in solution, from n.m.r. evidence.

Discussion

Natural enterolactone is racemic, although most other lignans of 2,3-dibenzylbutan-4-olide type are optically active, having the absolute configuration corresponding to matairesinol (8*R*,8′*R*). We therefore present all formulae in the (8*R*,8′*R*)

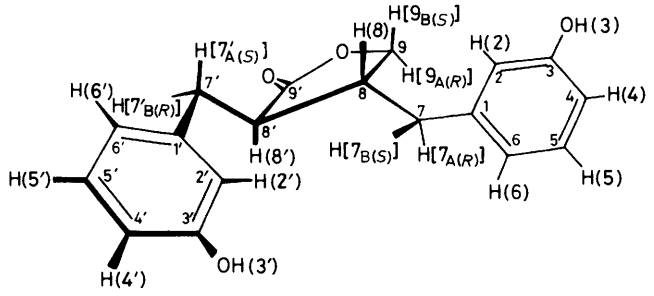


Figure 8. Preferred conformation of enterolactone in solution, as determined by n.m.r. [drawn for the (8*R*,8'*R*) configurations, with designation of individual protons: see text for explanations of subscripts]

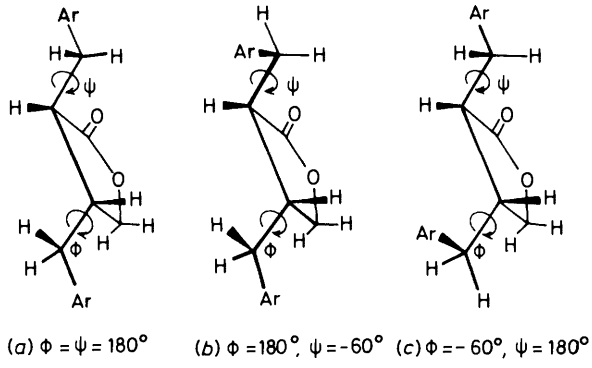


Figure 9. Proposed convention for designating torsion angles in *trans*-2,3-dibenzylbutan-4-olides

configuration in this paper. Having selected this enantiomer of enterolactone for discussion it is possible to distinguish unambiguously between the protons at each of the prochiral centres of C(7), C(7'), and C(9) by designating individual protons as either *pro-R* or *pro-S*.[28,29] For general purposes, including comparisons with n.m.r. data for other lignans, this seems more useful than the subscripts A and B used earlier in the present paper, which refer only to relative chemical shifts (see footnote * to aliphatic protons section), and might introduce ambiguities if used as labels for the protons themselves. Figure 8 illustrates the proton labelling based on prochirality, with the associated spectral assignments derived from n.O.e. data and *J* values.

Apart from flexing of the lactone ring, and associated changes in the C(7)–C(8)–C(8')–C(7') torsion angle, rotation about the bonds C(1)–C(7), C(7)–C(8), C(1')–C(7'), and C(7')–C(8') provide the main scope for conformation variations. Barriers to rotation of the aromatic rings with respect to the benzylic positions (rotation about 1–7 and 1'–7' bonds) should be small: n.O.e. data accordingly failed to distinguish between the protons at C(2) and C(6), or at C(2') and C(6'), supporting the view that rotation of the aromatic rings is rapid on the n.m.r. time-scale. The bonds (7–8 and 7'–8') linking the benzylic carbons to the lactone ring, however, would be expected to have relatively high barriers to rotation, disfavouring particularly the crowded rotamers in which the aryl and lactone rings are adjacent.

For convenient discussion of conformations, we have adopted a convention similar to that recommended [26,30] for the definition of torsion angles in a polypeptide chain. Figure 9 illustrates the three possible least crowded rotameric forms, designed respectively by their values of the torsion angles, φ and ψ. The 180°, −60° conformer (*b*) (and its enantiomer) are those which coexist in the crystal.

The coupling constants $J_{7_A,8}$ and $J_{7'_A,8'}$ are larger, respectively, than $J_{7_B,8}$ and $J_{7'_B,8'}$, suggesting that in solution the protons with ' A ' subscripts lie predominantly antiperiplanar to H(8) or H(8'). The n.O.e. data can be almost wholly accommodated by the 180°, −60° conformation (*b*) (Figure 9), corresponding to that found in the crystal, but not by either of the alternative uncrowded conformations. The observation of *negative* n.O.e.s in two cases [H(7'$_A$)→(8'), and H(9$_B$)→(7$_A$) and H(7$_B$)] implies that the proton spins in question interact in each case through an intermediary proton rather than directly through space. Conformers in which these pairs of protons would be close enough to interact directly must therefore be poorly populated. The benzylic group with primed locants, in particular, would appear to have a strong preference for its −60° conformation, since all the observed n.O.e.s involving this group can be explained in terms of its *pro-S* proton [H(7'$_A$)] being the only one of the 7'-pair which spends most of its time in a position close to the main bulk of the rest of the molecule, as well as showing a negative n.O.e. at H(8') when H(7'$_A$) was irradiated [Figure 6(c)].

Both benzylic protons at C(7), in contrast, exhibit *positive* n.O.e.s on irradiating H(9$_A$) or the more remote H(9$_B$), indicating that both H(7$_A$) and H(7$_B$) spend appreciable times close to H(9$_A$). Some freedom of rotation of this benzylic group between its preferred 180° conformation and the −60° conformation is implied, although the latter must be a relatively minor contributor.

In summary, all the evidence is compatible with the 180°, −60° conformation (*b*) being predominant in solution as well as being the form found in the crystal.

We propose the stereochemical conventions represented in Figures 8 and 9 for general adoption when discussing dibenzylbutane lignans.

Experimental

The n.m.r. spectra were carried out in the pulsed Fourier transform mode with internal deuterium lock, at 100 MHz (^{1}H) and 25.1 MHz (^{13}C) on a JEOL-FX-100 at Westfield College, at 200 (^{1}H) and 270 MHz (^{1}H) and 67.8 MHz (^{13}C) on a JEOL-FX-200 and FX-270, respectively, at JEOL (UK) Ltd., Grove Park, Colindale, London and at 400 MHz (^{1}H) and 100 MHz (^{13}C) on the University of London Intercollegiate Research Service (ULIRS) Bruker WH-400 at Queen Mary College.

Spectra were accumulated into 8K, 16K, and 32K data blocks with pulse widths varying from 30–180° flip angle depending upon the experiment being executed. Several resolution enhancement methods were used including convolution difference,[31] smoothing difference,[32] sine bell,[33] trapezoidal,[34] and Gaussian.[35] Simultaneous acquisition of blank and irradiated spectra for observation in difference mode was employed in order to minimise drift errors. Sample concentrations, solvents, and temperatures are as listed in Tables 1, 3, and 4. The ^{13}C n.m.r. spectrum of naturally occurring enterolactone (1a) was obtained using a microprobe (1.7 mm o.d.) with dual proton and carbon capability on a JEOL-FX-100 at 25.1 MHz (0.4 mg in 20 μl $CDCl_3$ + 10μl C_5D_5N; 300 K).

All results are quoted using standard notation: *J* in Hz, s = singlet, d = doublet *etc.*, δ in p.p.m. from internal Me_4Si.

X-*Ray Structure Determination*.—Crystals were obtained from chloroform. All *X*-ray measurements were made using a Nonius CAD4 diffractometer using previously described procedures.[36] The structure was solved *via* direct methods (MULTAN), developed by standard difference syntheses

including all H atoms, and refined by full-matrix least-squares using programs of the Nonius SDP system.

Crystal data. $C_{18}H_{18}O_4$, $M = 298.34$, monoclinic, $a = 14.274(2)$, $b = 10.065(2)$, $c = 11.165(1)$ Å, $\beta = 106.60$ (1)°, space group $P2/n$, $Z = 4$, $D_c = 1.28$ g cm^{-3}, μ(Cu-K_α) = 7.02 cm^{-1}, λ (Cu-K_α) 1.54178A, $F(000) = 632$.

Data collection. $\omega/2\theta$ scan, $\omega = 0.85 + 0.15 \tan\theta$, $3.0 < \theta < 70.0°$, ω scan speed 1.44–6.70 deg min^{-1}, $\sigma(I)/I$ required = 0.03 but $t_{max.} = 60$ s. 3244 reflections measured, 2915 unique, 2181 observed [$I > 1.5\ \sigma(I)$].

Structure refinement. C and O atoms anisotropic, H atoms isotropic. $R = 0.048$ (unit weights for the 2181 observed reflections). Final atomic positional parameters are given in Tables 5 and 6. Thermal parameters and values of F_O/F_c have been deposited as Supplementary Publication No. SUP 23812 (21 pp.).*

* For details of the Supplementary Publication Scheme see Instructions for Authors, *J. Chem. Soc., Perkin Trans. 1*, 1984, Issue 1.

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