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X-RAY CRYSTAL STRUCTURE AND ABSOLUTE CONFIGURATION OF KAEROPHYLLIN (CHAEROPHYLLIN)

KHADGA S. KHETWAL, SUBODH HARBOLA,

Department of Chemistry, Kumaon Univeristy, Nainital 263001, U.P., India

PING CAI, JOHN K. SNYDER,

Department of Chemistry, Boston University, Boston, Massachusetts 02215

M. GARY NEWTON,

X-Ray Diffraction Laboratory, Department of Chemistry,

S. WILLIAM PELLETIER, and BALAWANT S. JOSHI*

Institute for National Products Research and the Department of Chemistry, University of Georgia, Athens, Georgia 30602

ABSTRACT.—The structure of kaerophyllin [2], isolated from *Bupleurum lanceolatum*, has been confirmed by an X-ray crystallographic analysis. Accurate ^{1}H- and ^{13}C-nmr assignments have been made on the basis of COSY, long range homonuclear (long range COSY), heteronuclear (selective INEPT and FLOCK) scalar interactions, and nOe experiments. The absolute configuration of the lignan has been defined as α-(*trans*-3,4-dimethoxybenzylidene)-β-R-(3,4-methylenedioxybenzyl)-γ-butyrolactone.

Among the numerous types of plant-derived lignans, those with the 2,3-diaryl-butyrolactone skeleton [1] have potentially useful antitumor activity (1). Lignans of this kind are the precursors of the aryltetalin-type bioactive lignans. Podophyllotoxin, isolated from various species of *Podophyllum*, is an important starting material for the preparation of the clinically useful anticancer drugs etoposide and teniposide (2–4). A search for plants of the high altitude Himalayan glaciers was conducted with a view to discover new medicinal compounds. In this communication, we report accurate ^{1}H- and ^{13}C-nmr chemical shift assignments, X-ray crystal structure determination, and absolute configuration of the lignan, kaerophyllin (chaerophyllin) [2], isolated from *Bupleurum lanceolatum* (Umbelliferae) (5,6).

RESULTS AND DISCUSSION

A colorless crystalline compound 2, mp 150°, was obtained from the aerial parts of *B. lanceolatum*, collected at an altitude of ca. 3500 m, in Kumaon Himalayan glaciers. The eims ([M]$^+$, m/z 368) and two major fragments at m/z 233 ($C_{13}H_{13}O_4$) and m/z 135 ($C_8H_7O_2$) indicated the presence of a methylenedioxybenzyl group and suggested the molecular formula $C_{21}H_{20}O_6$ for the lignan. The ^{1}H- and the ^{13}C-nmr spectra (Tables 1 and 2) were consistent with the proposed structure 2; a literature search indicated that

1

2

3
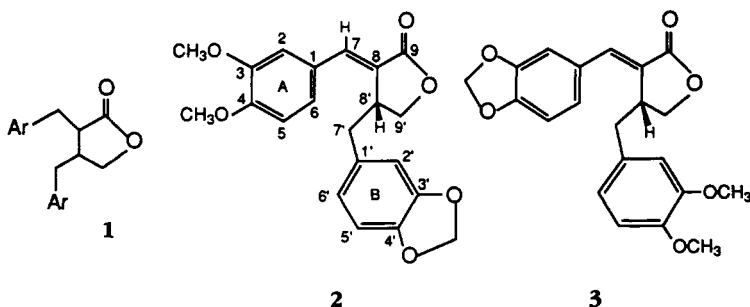
the structure **2** has been assigned to kaerophyllin (chaerophyllin), isolated from *Chaerophyllum maculatum* (7). A lignan having the structure **2** designated as chisulactone mp 108–111°, [α]D −72.6° (wrongly given the molecular formula $C_{21}H_{22}O_6$), was isolated from *Polygala chinensis* (1,8,9).

In the previous structural proof of kaerophyllin (7), location of the two MeO groups and the methylenedioxy group at 3, 4 and 3′, 4′ positions, respectively, rested entirely

TABLE 1. ^{1}H- and ^{13}C-nmr Chemical Shifts of **2**.

Position	^{1}H (CDCl$_3$)	^{1}H (C$_6$D$_6$)	^{13}C (CDCl$_3$)	^{13}C (C$_6$D$_6$)
1	—	—	126.8	127.4[a]
2	7.03 (d, J = 2.0 Hz)	6.73 (d, J = 1.9 Hz)	112.9	113.8
3	—	—	150.7	151.4[b]
4	—	—	149.1	150.0
5	6.93 (d, J = 8.4 Hz)	6.43 (d, J = 8.3 Hz)[c]	111.2	111.8
6	7.20 (dd, J = 8.4, 2.0 Hz)	6.93 (dd, J = 8.3, 1.9 Hz)	123.6	123.7
7	7.51 (d, J = 1.9 Hz)[d]	7.77 (br s)	137.4	136.9
8	—	—	125.6	126.7[a]
9	—	—	172.6	171.8
1′	—	—	131.5	132.1[a]
2′	6.63 (d, J = 1.6 Hz)	6.44 (br s)[c]	109.0	109.3
3′	—	—	148.0[e]	148.4[b]
4′	—	—	146.5[e]	146.9
5′	6.71 (d, J = 7.8 Hz)	6.53 (d, J = 7.8 Hz)	108.5	108.5
6′	6.61 (dd, J = 7.8, 1.6 Hz)	6.23 (dd, J = 7.8, 1.1 Hz)	121.9	122.2
7′a	3.02 (dd, J = 14.4, 4.2 Hz)	2.74 (dd, J = 14.4, 4.3 Hz)	37.4[f]	37.3[f]
7′b	2.59 (dd, J = 14.4, 10.3 Hz)	2.17 (dd, J = 14.4, 10.3 Hz)	—	—
8′	3.80 (m)	3.24 (m)	39.7	39.6
9′a	4.25 (m, 2H)[g]	3.82 (dd, J = 9.0, 1.4 Hz)	69.5	69.0
9′b	—	3.65 (dd, J = 9.0, 7.1 Hz)	—	—
3-OMe . . .	3.89 (s)	3.38 (s)	56.00[h]	55.4[i]
4-OMe . . .	3.92 (s)	3.30 (s)	55.95[h]	55.6[i]
-OCH$_2$O-	5.91 (AB, J_{AB} = 1.4 Hz)	5.28 (br s)	101.1	101.0

[a]The assignments of C-1, C-8, and C-1′ in C_6D_6 are based upon the unambiguous assignments in $CDCl_3$ and not upon directly observed long range heteronuclear couplings in this solvent (i.e., C-1, C-8, and C-1′ were distinguished in $CDCl_3$, not in C_6D_6).

[b]The assignments of C-3 and C-3′ in C_6D_6 are based upon the unambiguous assignments in $CDCl_3$ and not upon directly observed long range heteronuclear couplings in this solvent.

[c]Overlap between H-2′ and H-5 prevented resolution of the *m*-coupling between H-2′ and H-6′, ($^4J_{2',6'}$ = 1.1 Hz) and the *o*-coupling ($^3J_{5,6}$ = 8.3 Hz) between H-5 and H-6. These coupling constants were therefore determined from their respective coupled partners (H-6′ and H-6).

[d]Coupling (1.9 Hz) with H-8′ was confirmed by decoupling experiments. Long range coupling was detected in the LRCOSY experiment to several protons (see Table 2).

[e]The assignments of C-3′ and C-4′ in $CDCl_3$ are based upon the unambiguous assignments in C_6D_6 and not upon directly observed long range heteronuclear couplings in this solvent (i.e. C-3′ and C-4′ were distinguished in C_6D_6, not $CDCl_3$).

[f]Correlations between the H-7′ methylene protons and C-7′ were not observed in the HETCOR spectrum. Nevertheless, this is the only reasonable assignment even without a DEPT or APT experiment since C-8′ could be directly assigned from the HETCOR spectrum.

[g]Protons 9′a, 9′b and 8′ formed an ABX system with |$\nu_A - \nu_B$| being very small. Analysis of such a system to extract J_{AB}, J_{AX}, and J_{BX}, as well as δ_A and δ_B, was not possible, and the analysis was further complicated by the additional coupling of H-8′ to the C-7′ methylene protons. The diastereotopic C-9′ methylene protons were cleanly resolved in C_6H_6-d_6.

[h]The assignment of these two resonances may be interchanged.

[i]These assignments were confirmed by a HETCOR experiment with resolution in the proton dimension enhanced by increasing the number of increments to 512 and reducing the spectral window in the proton dimension to 2339 Hz.

on the ms fragment observed at m/z 135, attributed to methylenedioxybenzyl radical and absence of a peak at m/z 151. The absolute stereochemistry of kaerophyllin was not determined. Also, the aromatic protons of the A and B rings were not assigned, and the ^{13}C-nmr spectral assignments appeared to be arbitrary. We therefore took up a detailed nmr study in order to make definite ^{1}H- and ^{13}C-nmr assignments and to determine the absolute configuration. As there has been a diversity of systems in numbering the atoms of lignans, the latest numbering system suggested by Ayres and Loike (1) is used.

NMR ASSIGNMENTS.—The major task of any significance for the assignment of the ^{1}H and ^{13}C resonances of kaerophyllin was to distinguish the two 1,3,4-trisubstituted (3,4-dioxygenated) aromatic rings and to assign the nonprotonated carbons. Distinguishing the aryl systems, which were both easily discerned in the COSY spectra, meant correlating dimethoxyaryl spins (either proton or carbon) primarily with the β-position spins (either H-7 or C-7) of the α,β-unsaturated lactone, and correlating dioxymethylene-substituted aryl spins (either proton or carbon) with 7'-, 8'-, or 9'-position spins. These correlations could be directly observed either through scalar or dipolar couplings and would also confirm the structure assignment of the compound as kaerophyllin [2] (7) rather than the isomer 3. The isomer 3 was originally obtained as a pyrolysis product of helianthoidin, a lignan esterified to angelic acid, isolated from *Heliotropis scabara* (10). Assignment of nonprotonated carbons would be accomplished through the observation of long range heteronuclear couplings (^2J or ^3J) using either the 1D selective INEPT (11,12) or 2D FLOCK (13) sequences.

Complete proton and carbon assignments were done in both CDCl$_3$ and C$_6$D$_6$ (Table 1). In CDCl$_3$, the C-9' methylene protons were nearly magnetically equivalent, forming a tight ABX system with H-8'. While analysis of such a system to extract J_{AB}, J_{AX}, and J_{BX}, as well as δ_A and δ_B is possible (14), this analysis would require that all six lines of the AB portion of the ABX system be unambiguously observed. This was not possible in the present case, and the analysis was further complicated by the additional coupling of H-8' to the C-7' methylene protons. All other proton resonances were well-resolved in CDCl$_3$ and were easily assigned.

TABLE 2.   Useful nOe's and Long Range Scalar Couplings of 2.

Solvent	^{1}H nOe's[a]	Homonuclear ^{1}H-^{1}H Long Range Scalar Couplings[b]	Heteronuclear ^{13}C-^{1}H Long Range Scalar Couplings
CDCl$_3$	H-2–3-OMe (NOESY) H$_b$-7–H-2', H-6', H-8' (DNOE) H-8'–H-9'^{c} (NOESY) H-8'–H-2, H-6, H-2 (DNOE)	H-2–H-6, H-7, 3-OMe H-5–H-7, 4-OMe H-2'–H-6' H-7–H-8', H-9'^{c} H$_a$-7'–H-9'^{c}	H-2–C-3, C-4, C-6 (FLOCK) H-7–C-6 (FLOCK) H-5–C-1 (FLOCK) H-2'–C-6' (SINEPT) H-8'–C-8, C-9, C-1' (SINEPT) H-9'^{d}–C-8, C-9 (SINEPT) 3-OMe–C-3 (FLOCK) 4-OMe–C-4 (FLOCK)
C$_6$D$_6$	H-2–3-OMe, H-8' (NOESY) H-5–4-OMe (NOESY) H-6–H-2', H-8' (NOESY) H$_a$-7'–H-2, H-6, H-2', H-6' (DNOE) H$_b$-7'–H-2', H-6', H$_a$-9' (DNOE) H-8'–H$_b$-9' (NOESY)	H-2–H-5, H-6, H-7, 3-OMe H-5–H-7, 4-OMe H-6–H-7 H-7–H$_a$-9' H-2'–H-5', H-6', H$_b$-7' H$_a$-7'–H$_b$-9' H$_b$-7'–H$_a$-9'	H-6–C-4 (SINEPT) H-6'–C-4' (SINEPT)

[a]NOe's observed between vicinal or geminal protons are not included in this list; nOe's are not listed in duplicate for each pair, but only by lowest numbered proton (non-primed before primed numbers) for NOESY-detected nOe's, or by saturated resonance for DnOe-detected nOe's.

[b]List includes 4-bond or longer couplings obtained from LRCOSY spectrum, $\Delta = 300$ ms. Couplings are not listed in duplicate for each pair, but are only listed by lowest numbered proton (non-primed before primed numbers).

[c]Due to overlap between H$_a$-9' and H$_b$-9' in CDCl$_3$, it was not possible to distinguish off-diagonal elements to these protons unambiguously.

[d]Due to overlap between H$_a$-9' and H$_b$-9' in CDCl$_3$, both resonances could be simultaneously saturated.

The two different aryl spin systems were distinguished in both the long range COSY (LRCOSY, D = 300 ms) and NOESY spectra (Table 2). In the LRCOSY spectra, H-7 (δ 7.51, d, J = 1.9 Hz) showed coupling ($^4J_{2,7}$) with the *m*-coupled doublet, (H-2, δ 7.03, d, J = 2.0 Hz) and the *o*-coupled doublet ($^5J_{5,7}$, H-5, δ 6.93, d, J = 8.4 Hz), the latter of which was *o*-coupled to H-6 (δ 7.20, dd, J = 8.4, 2.0 Hz). Additional weak couplings with H-7 included H-8' and one or both of the C-9' methylene protons. The coupling with H-8' (J = 1.9 Hz) was responsible for the doublet multiplicity of H-7 observed in the 1D proton spectrum, as confirmed by decoupling experiments. The coupling with H-9' could not be specifically assigned to either H_a-9' or H_b-9' due to the near overlap of these tightly coupled protons. Interestingly, under the conditions of this LRCOSY experiment, coupling was not observed between H-6 and H-7, nor was benzylic coupling observed between H-7' and either H-2' or H-6'. The methoxyl groups were in turn coupled through five bonds to the aryl protons ortho to the methoxyl substitution sites: the C-3 methoxyl group showed long range coupling with H-2, and the C-4 methoxyl group coupled with H-5.

The NOESY spectrum confirmed the assignment of the aryl proton spin systems, as well as the assignment of the two methoxyl proton resonances. An nOe was observed between the C-3 methoxyl group and H-2. In a difference nOe (DnOe) experiment, nOe's were also seen between H_b-7' and both H-2' and H-6'. While these latter nOe's support the location of the aryl ring bearing the dioxymethylene group at C-7', nOe's were also observed between H_a-7' and protons on the dimethoxylated aryl ring (H-2 and H-6) as well as with protons on the aryl ring bearing the dioxymethylene group.

With the distinction of the two aryl proton systems, all protons for **2** could be assigned with the exception of the two C-9' methylene protons, owing to their near magnetic equivalence. In addition, the *pro-R* and *pro-S* C-7' methylene protons were also not distinguished, though this would have been possible with a more detailed conformational analysis. Nevertheless, the significant difference in the couplings between H-8' and the two C-7' methylene protons suggested a dominant conformation about the C-7'/C-8' bond with a dihedral angle between H_b-7' and H-8' of 180° and a dihedral angle between H_a-7' and H-8' of 60°. This was confirmed in the X-ray study, which showed the corresponding torsional angles of 178.78° and 56.6°, respectively. Assuming the C-8' carbon to be an *R* center as drawn, this would suggest that H_a-7' is the *pro-S* proton and H_b-7' is the *pro-R* proton with the dominant conformation of **2** as shown in Figure 1. This conformation is in accord with the more extensive nOe's obtained in C_6D_6.

As the diastereotopic C-9' methylene protons were cleanly resolved in C_6D_6, complete proton assignments were also undertaken in this solvent. The H-2' and H-5 aromatic protons, however, were overlapped in C_6D_6. The protons of the two aryl spin systems were distinguished on the basis of the long range scalar couplings observed in an LRCOSY experiment (Δ = 300 ms), as well as from dipolar couplings recorded in NOESY and DnOe spectra, analogous to those observed in $CDCl_3$ (Table 2). Thus, in the LRCOSY experiment it was observed that H-7 coupled through four bonds with both H-2 and H-6, as well as through five bonds with H-5. Additional weak coupling was observed between H-7 and H_a-9' but not with H-8' (as observed in $CDCl_3$). Four-bond coupling was also detected in this experiment between H_b-7' and the *m*-coupled H-2', which was a member of the other 1,2,4-trisubstituted aryl system along with H-5' and H-6'. As observed in $CDCl_3$, the methoxyl groups were coupled through five bonds to the aryl protons ortho to the methoxyl substitution sites: the C-3 methoxyl group (δ 3.38, s) coupled with H-2, and the C-4 methoxyl group (δ 3.30, s) coupled with H-5. Dipolar couplings (nOe's) observed between H-2 and the C-3 methoxyl group and between H-5 and the C-4 methoxyl group confirmed the assignment of the
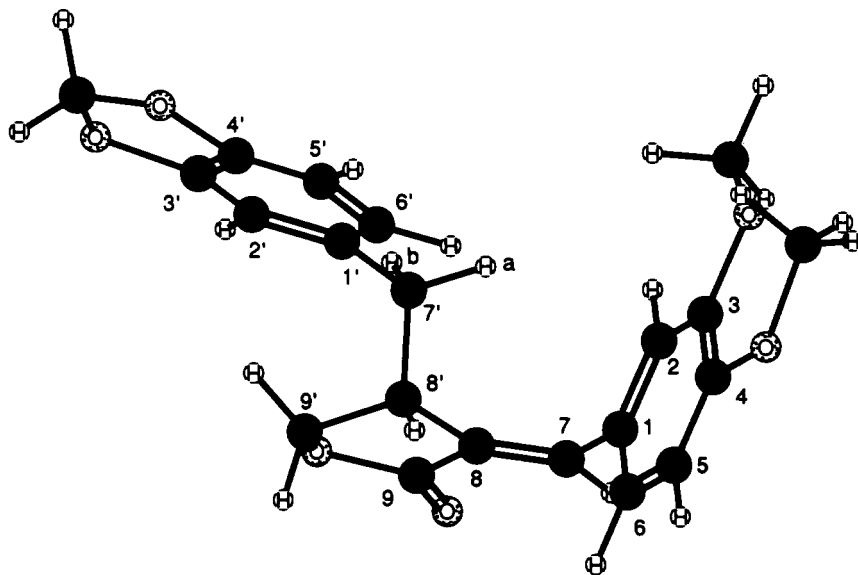
FIGURE 1.   The dominant conformation of **2**.

two aryl proton spin systems as well as the distinction of the two methoxyl proton reso-
nances. Unlike the relative shifts in CDCl$_3$, where the aryl protons of the dimethoxyl-
ated ring were all upfield from those of the dioxymethylated ring, in C$_6$D$_6$ H-5 was
shifted considerably upfield and overlapped with H-2'.

The C-9' methylene protons could also be assigned both from the couplings with
H-8' and from nOe's observed in DnOe and NOESY spectra (Table 2). One of the C-9'
methylene protons (H$_b$-9', δ 3.65) had relatively large coupling with H-8' (7.1 Hz)
while H$_a$-9' (δ 3.82) showed only weak coupling with H-8' (1.4 Hz), suggesting that
the former proton was *cis* to H-8' with a 0° dihedral angle due to the rigidity of the γ-
lactone ring. These assignments were supported by the nOe's: H$_b$-9' showed an nOe
with H-8' in the NOESY spectrum, while H$_a$-9' had an nOe with H$_b$-7'.

With all the proton resonances assigned, the assignment of the protonated carbon
resonances was routine from the heteronuclear COSY (HETCOR) spectrum. Given the
simplicity of the molecule, the one-bond carbon multiplicities were deduced directly
from the HETCOR spectrum, and an APT or DEPT experiment was unnecessary. The
only correlations which were not observed in the HETCOR spectrum were between C-
7' and its methylene protons, presumably due to the strong geminal coupling (15,16).
While these correlations undoubtedly would have appeared in Reynolds' fixed evolu-
tion HETCOR experiment (15,16), the assignment of C-7' was unambiguous since
this was the sole sp^3 hybridized carbon in the carbon spectrum which did not correlate
with any protons in the HETCOR experiment. The carbon resonances of the two
methoxyl groups were nearly equivalent in CDCl$_3$ (δ 56.002 and 55.954, Δδ = 15 Hz)
and could not be distinguished under the conditions of the HETCOR experiment
(SW2 = 20000 Hz, NP = 512, zero-filled to 1024, digital resolution in the carbon di-
mension DR2 = 39 Hz; SW1 = 4000, NI = 256, digital resolution in the proton di-
mension DR1 = 31 Hz). The chemical shift difference between the proton resonances of
these methoxyl groups (Δδ = 14 Hz) was also insufficient for resolution of the cross
peaks under these conditions. By repeating the HETCOR experiment in C$_6$D$_6$, in

which the methoxyl carbons were slightly better resolved (δ 55.565 and 55.354, $\Delta\delta = 21$ Hz) under conditions for better resolution in the proton dimension (SW1 = 2339 Hz, NI = 512, for a digital resolution in the proton dimension of 9 Hz; the chemical shift difference between the proton resonances of the methoxyl groups in C_6D_6 was 29 Hz), the distinction of the methoxyl carbons was possible.

The nonprotonated carbons were assigned by the observation of long range (two- and three-bond) heteronuclear couplings in both $CDCl_3$ and C_6D_6 (Table 2). A FLOCK experiment in $CDCl_3$ distinguished C-3 (δ 150.7) and C-4 (δ 149.1) by couplings with their respective methoxyl protons, while selective INEPT experiments distinguished C-8, C-1', and C-1 by three-bond couplings with H-9', H-8', and H-5, respectively. The assignment of C-9, obvious from its chemical shift, was confirmed by the observation of long range coupling with H-8' and H-9' in a selective INEPT experiment.

The rigidity of the γ-lactone ring severely restricts the degrees of conformational freedom available to **2**; only rotation about the C-7'/C-8' bond and rotations of the aryl rings are conceivable. The nOe's of H-8' with both H-2 and H-6, as well as those of H$_a$-7' and H$_b$-7' with both H-2' and H-6', indicated that the phenyl rings are relatively free to rotate. The great difference in the couplings between H-8' with the two C-7' methylene protons suggested that a single, dominant conformation about the C-7'/C-8' bond exists (17–19). This conformation in C_6D_6 could be deduced from the ^3J constants and the observed nOe's. The large coupling $^3J_{7'b,8'}$ (10.3 Hz) suggested a 180° dihedral angle between these two protons with a 60° dihedral angle between H$_a$-7'/H-8' ($^3J_{7'a,8'}$ = 4.3 Hz). This dominant conformation (Figure 1) was supported by nOe's between H$_a$-7' with both H-2 and H-6, H$_b$-7' with H$_a$-9', and H-2' with H-6'. The nOe's of H$_a$-7' and H$_b$-7' were observed in DnOe spectra, while the H-6/H-2' nOe was seen in the NOESY spectrum. While the lack of nOe's cannot be taken as definitive evidence, no nOe's were observed between H$_a$-7' and either of the C-9' methylene protons, nor was an nOe observed between H$_b$-7' and H$_b$-9'. These nOe's would not be expected if **2** remained predominantly in the conformation shown in Figure 1. The dominant conformation of **2** in space filling model (ChemDraw 3D), indicates steric interactions between H-2/H-6 and the C-7' methylene group, preventing the dimethoxylated aryl ring from achieving optimal overlap with the α,β-unsaturated lactone.

ABSOLUTE CONFIGURATION.—On the basis of the ord and cd of a number of lignans of established structures, it is possible to determine the absolute stereochemistry of newly isolated lignans (20,21). Burden *et al*. (10) have used this method in arriving at the absolute configuration of helianthoidin and its derivatives. The absolute stereochemistry of the lignan **3**, mp 129–130°, [α]D −83.3°, obtained by pyrolysis of helianthoidin which has the 8S,8'S configuration, was assigned the (−)-R configuration by comparison of the cd and ord curves with (−)-(R)-hibalactone. The absolute stereochemistry of hiblactone was in turn established by chemical correlation with 3,4-dimethoxyphenylalanine (22). Isolation of **3** as a natural product from *P. chinensis* was first reported by Ghosal *et al*. (8). They noted that this lignan designated as suchilactone was the same compound as the lignan **3** obtained by Burden *et al*. (10), but without any comments or reporting the optical rotation, they wrongly assigned it the S configuration. Subsequently, a lignan, mp 129°, [α]D +87°, having the structure **3** (X-ray) was isolated from *Jatropha gossypifolia* (23). Based on Ghosal's erroneous stereochemistry of suchilactone, Chatterjee *et al*. (23) concluded that their lignan had the R configuration, which in fact is the (+)-S enantiomer of **3**. The lignan **2** isolated by us from *B. lanceolatum* showed a negative split Cotton effect (Figure 2), indicating the stereochemistry at C-8' as R. As both the enantiomers of **3** exist in nature, the assignment of the absolute stereochemistry of kaerophyllin becomes significant. The structure of kaerophyllin
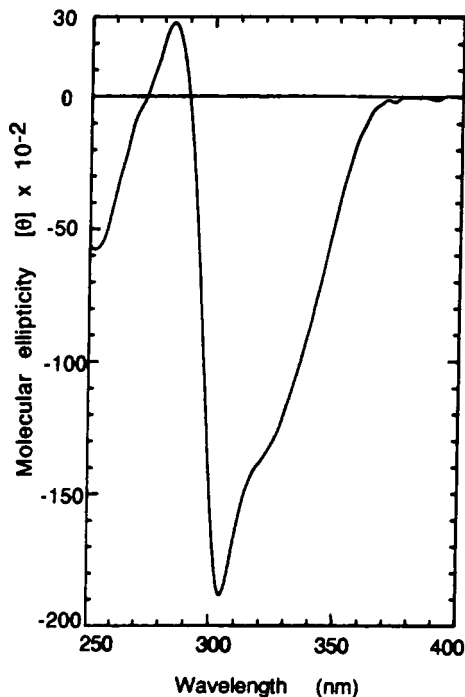
FIGURE 2.   Cd data of **2** in CHCl$_3$.

can be defined as α-(*trans*-3,4-dimethoxybenzylidene)-β-*R*-(3,4-methylenedioxyben-zyl)-γ-butyrolactone.

X-RAY CRYSTAL STRUCTURE.—As we were unable to obtain an authentic sample of kaerophyllin, we decided to carry out an X-ray crystallographic analysis to establish the structure of the lignan isolated by us. This study confirmed the structure, and Figure 3 shows the ORTEP drawing of kaerophyllin. All bond distances and angles are within the normal ranges expected for the bond type. The twelve aromatic C(sp^2)–C(sp^2) distances range from 1.357 to 1.400 Å with an average distance of 1.386 Å. The four C(sp^2)-O ether distances average 1.374 Å, and the four C(sp^3)-O ether distances average 1.420 Å. The only intermolecular contact (between non-hydrogen atoms) less than 3.30 Å is O-4–C-2′ with a distance of 3.207(3) Å. The absolute stereochemistry of kaerophyllin as shown in Figure 3 was drawn to conform to the *R* configuration, and the C-7–C-8 double bond was determined to be in an *E* configuration. The phenyl rings are nearly coplanar with a twist angle of 12.8(3)°. It is evident that no bonding exists between C-6 and C-7′; the distance between these atoms is 3.719(5) Å.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The nmr spectra were obtained on a Varian XL-400 spectrometer (93.94 kG, 400 MHz for ^{1}H, 100 MHz for ^{13}C). The pulse sequences employed in the 1D and 2D nmr experiments were the standard Varian software, version 6.1c, except the FLOCK pulse sequence which was added to the Varian nmr pulse sequence library based on Reynolds' program (13). Tlc (Si gel) plates were developed in CHCl$_3$-MeOH (65:35), and the spots were visualized either in long-range uv light (365 nm) or by spraying with 15% H$_2$SO$_4$-Ac$_2$O (1:3) solution and heating at 100° for 15 min. Eims was recorded (direct inlet) on Finnigan Quadrupole 4023 instrument at 70 eV.
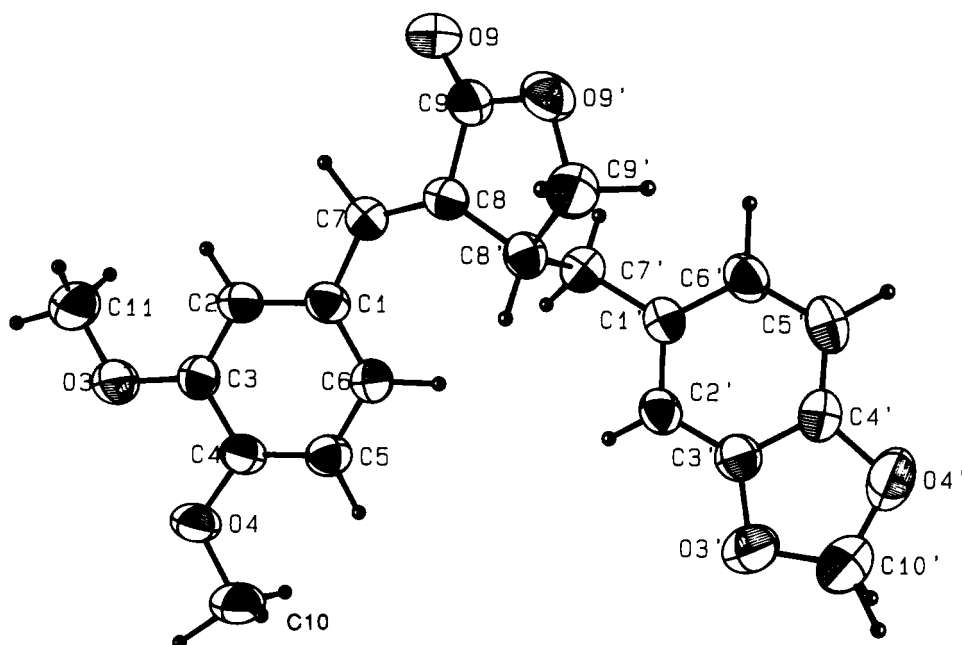
FIGURE 3.   ORTEP drawing of **2**.

PLANT MATERIAL.—Aerial parts of *B. lanceolatum* were collected in the high altitude regions (3500 m) of Kumaon Himalayan glaciers, India. The plant was authenticated in the Botany Department of Kumaon University. A voucher specimen has been deposited in the herbarium of Kumaon University.

EXTRACTION AND ISOLATION OF KAEROPHYLLIN [**2**].—Shade-dried aerial parts of the plant were powdered and extracted in a Soxhlet apparatus with 90% EtOH. The EtOH extract was concentrated in vacuo and partitioned with $CHCl_3$-H_2O (1:1). The $CHCl_3$ layer was separated, concentrated, and extracted with petroleum ether (60–80°). The petroleum ether extract was chromatographed on Si gel and eluted with petroleum ether and increasing amounts of C_6H_6. Petroleum ether-C_6H_6 (90:10) afforded a

TABLE 3.   Crystal Structure Data for Kaerophyllin [**2**].

Crystal shape	thin needle
Crystal dimensions	$0.15 \times 0.15 \times 0.5$ mm
Molecular formula	$C_{21}H_{20}O_6$
Molecular weight	368.39
a	7.616(2) Å
b	8.766(2) Å
c	13.850(2) Å
α	90.0°
β	102.42(2)°
γ	90.0°
V	902.97(4) Å³
F(000)	388
μ(CuK$_\alpha$)	7.8 cm^{-1}
λ(CuK$_\alpha$)	1.54184 Å
D$_{calc}$	1.355 g/cm³
Z	2
θ range	2–75°
Number of reflections measured . . .	2029
Number of reflections used ($F_0 > 3\sigma$) .	1466
R$_w$	0.048
Space group	$P2_1$ (#4)

TABLE 4.   Positional Parameters and Their Estimated Standard Deviations.

Atom	x	y	z	B(A2)
O-3	0.9971(3)	0.4353(3)	0.4261(1)	5.30(5)
O-3'	0.4110(3)	0.2202(4)	−0.3017(2)	7.16(7)
O-4	0.6951(3)	0.3143(3)	0.3418(1)	4.85(4)
O-4'	0.4815(3)	0.2508(4)	−0.4554(2)	6.23(6)
O-9	1.4589(3)	0.6340(4)	0.0207(2)	5.70(6)
O-9'	1.3442(2)	0.4785(3)	−0.1051(1)	4.64(4)
C-1	1.0341(3)	0.4720(4)	0.1644(2)	3.51(5)
C-1'	0.8094(3)	0.4630(3)	−0.2185(2)	3.32(5)
C-2'	0.6621(3)	0.3743(4)	−0.2093(2)	3.64(5)
C-2	1.0786(4)	0.4821(4)	0.2677(2)	3.88(6)
C-3	0.9621(4)	0.4306(4)	0.3248(2)	3.87(6)
C-3'	0.5618(4)	0.3095(4)	−0.2931(2)	3.96(6)
C-4	0.7968(4)	0.3669(4)	0.2785(2)	3.74(5)
C-4'	0.6030(4)	0.3294(4)	−0.3841(2)	4.07(6)
C-5	0.7483(4)	0.3627(4)	0.1772(2)	3.78(5)
C-5'	0.7454(4)	0.4141(5)	−0.3969(2)	4.63(7)
C-6	0.8652(4)	0.415	0.1203(2)	3.70(5)
C-6'	0.8484(4)	0.4816(4)	−0.3121(2)	4.19(6)
C-7'	0.9269(4)	0.5314(4)	−0.1285(2)	3.74(6)
C-7	1.1715(4)	0.5182(4)	0.1111(2)	3.61(5)
C-8	1.1823(3)	0.4946(4)	0.0166(2)	3.40(5)
C-8'	1.0619(3)	0.4167(4)	−0.0695(2)	3.38(5)
C-9'	1.2013(4)	0.3657(5)	−0.1272(2)	4.41(6)
C-9	1.3426(4)	0.5449(4)	−0.0180(2)	4.06(6)
C-10'	0.3677(6)	0.1748(6)	−0.4013(3)	7.5(1)
C-10	0.5301(4)	0.2387(5)	0.2972(2)	5.90(8)
C-11	1.1453(5)	0.5230(6)	0.4746(2)	6.22(9)
H-2'	0.630(4)	0.367(4)	−0.149(2)	4.6ᵃ
H-2	1.195(4)	0.524(4)	0.302(2)	4.6ᵃ
H-5'	0.784(4)	0.419(4)	−0.462(2)	5.4ᵃ
H-5	0.624(4)	0.321(4)	0.147(2)	5.1ᵃ
H-6'	0.970(5)	0.549(5)	−0.320(3)	6.6ᵃ
H-6	0.824(3)	0.410(4)	0.042(2)	4.1ᵃ
H_b-7'	0.989(5)	0.614(5)	−0.150(2)	6.8ᵃ
H-7	1.292(4)	0.564(5)	0.155(2)	6.1ᵃ
H_a-7'	0.842(3)	0.568(3)	−0.082(2)	2.6ᵃ
H-8'	0.991(5)	0.330(6)	−0.046(3)	8.5ᵃ
H_a-9'	1.280(6)	0.259(7)	−0.099(3)	11.3ᵃ
H_b-9'	1.166(4)	0.368(5)	−0.205(2)	6.6ᵃ
H_a-10	0.489(4)	0.207(5)	0.371(2)	6.8ᵃ
H_b-10	0.447(6)	0.334(6)	0.256(4)	10.8ᵃ
H_c-10	0.555(5)	0.163(6)	0.257(3)	9.2ᵃ
H_a-10'	0.246(6)	0.197(9)	−0.433(4)	14.2ᵃ
H_b-10'	0.319(7)	0.049(9)	−0.426(5)	15.4ᵃ
H_c-11	1.138(6)	0.632(6)	0.449(3)	9.5ᵃ
H_a-11	1.144(4)	0.519(5)	0.550(3)	6.6ᵃ
H_b-11	1.269(5)	0.471(6)	0.468(3)	9.1ᵃ

ᵃThese atoms were refined isotropically. Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as: $(4/3)$* $[a^2$*$B(1,1) + b^2$*$B(2,2) + c^2$*$B(3,3) + ab(\cos \gamma)$*$B(1,2) + ac(\cos \beta)$*$B(1,3) + bc(\cos \alpha)$*$B(2,3)]$.

fraction which after repeated crystallizations from hexane gave colorless silky needles: mp 150°; uv (MeOH) λ max 234, 294, 329 nm; [α]D −91.8° (c = 0.39, CHCl₃); ir (KBr) ν max 3040, 2940, 2960, 2880, 2810, 2750, 1765, 1740, 1625, 1575, 1560, 1490, 1470, 1445, 1420, 1400, 1240, 1200, 922

cm^{-1}; eims *m/z* [M]$^+$ 368 (10%) (calcd for $C_{21}H_{20}O_6$, 368), [M − OMe]$^+$ 337 (2), 233 (80), 219 (1), 205 (10), 190 (5), 177 (40), 146 (14), 135 (100); ^{1}H and ^{13}C nmr see Tables 1 and 2.

X-RAY DATA FOR **2**[1].—A crystal of kaerophyllin was fixed in a random orientation on a glass fiber and mounted on an Enrauf-Nonius CAD-4 diffractometer equipped with a graphite crystal monochromator. Cell dimensions (Table 3) were determined by least squares refinement of the angular positions of 25 independent reflections in the 15–25° θ range during the normal alignment procedure. A total of 2029 reflections were collected over a θ range of 2–75° using ω–2θ technique with a variable scan width and scan range. Systematic absences indicated space group *P2₁* (#4). Because of the low value of the absorption coefficient, the data were not corrected for absorption. After Lorentz-polarization correction, averaging redundant data, and eliminating systematic absences, a total of 1466 reflections ($F_0 > 3\sigma$) were considered observed and unique and were used in the structural analysis.

The structural analysis was performed on a VAX 750 using MolEN structure analysis program system (24). The structure was solved using SIR88 (25) with 8 symbols and using the seminvariant and MESS options. All non-hydrogen atoms were located in several difference Fourier maps and refined by full-matrix least-squares, first isotropically, then anisotropically. Some hydrogen positions could be located from difference Fourier maps, and some hydrogen positions were calculated. All hydrogen atoms, with isotropic thermal parameters fixed, were refined along with positional and thermal parameters of non-hydrogen atoms via full matrix least squares to yield the final structure. The final unweighted R value was 0.037. Values for positional parameters and their estimated standard deviations for kaerophyllin are given in Table 4. Bond lengths and angles are all within a normal range and exhibit no unusual features.

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[1]Atomic coordinates for this compound have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

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