Tetrahedron Vol. 36, pp. 3489 to 3493
0040 4020/80 1215 3489 $02.00/0

THE STRUCTURE OF CLEOMEOLIDE, AN UNUSUAL BICYCLIC DITERPENE FROM *CLEOME VISCOSA* L. (CAPPARACEAE)

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(*Received in USA 6 November* 1979)

Abstract—A bicyclic diterpene, cleomeolide (**1**), $C_{20}H_{30}O_3$, has been isolated from *Cleome viscosa* L. (Capparaceae), and its stereostructure established by chemical, spectral and X-ray crystallographic means. Crystals of **1** belong to space group $P2_1$, with a = 10.329(2)Å, b = 12.468(3)Å, c = 7.356(2)Å, β = 109.98°, and Z = 2. The structure was solved by a multiple solution procedure and refined by full matrix least-squares to give R = 0.041 and wR = 0.051. Oxidation of **1** gave the ketone **4**, which on treatment with methanolic KOH led to a facile transannular reaction to give the lactone **6**.

The macrocyclic diterpenes cembrene[1] and casbene[2] may be regarded as the prototypes of a variety of natural products, many of which show pronounced biological activity.[3] Examples of these compounds are the esters of phorbol (cocarcinogens),[4] jatrophone (antileukemic),[5] gnidimacrin (antileukemic),[6] the *Pimelea* factors (irritants),[7] asperdiol (antileukemic),[8] taxol (antitumor, microtubule promotion),[9] and the kansuinines A and B (analgesic).[10] In continuation of our research[11] in this area, we report the isolation and structural characterization of cleomeolide (**1**), a bicyclic diterpene with a skeleton related to that found in verticillol,[12] and also discuss some interesting reactions encountered during our structural investigation.

Cleomeolide (**1**), $C_{20}H_{30}O_3$, m.p. 216–217°, was isolated as colourless crystals from a benzene extract of the leaves and twigs of *Cleome viscosa* (syn. *C. icosandra*, *Polanisia viscosa*, Capparaceae),[13] and had IR (KBr, cm^{-1}) absorptions indicative of OH (3435), exocyclic methylene (1640, 900), and α,β-unsaturated lactone (1683, 1628) groups. The single maximum in the UV spectrum at 214 nm (ε 4800) supported the presence of the latter functionality. The ^{1}H NMR spectrum of **1** displayed signals attributable to a secondary Me group (0.86, d, J = 7 Hz), a quaternary Me group (1.00, s), a Me group on a fully substituted C atom bearing an oxygen function (1.35, s), a methine proton of a secondary alcohol (4.53, br d, J = 12 Hz), a terminal methylene group (4.80 and 4.90, each a d, J = 2 Hz), and a vinyl proton (6.20, m). From the preceding spectral data and the molecular formula cleomeolide (**1**) is a bicarbocyclic diterpene. Additional structural information was obtained from the Fourier transform ^{13}C NMR spectrum (Table 1), which confirmed the presence of three Me groups (q at 15.8, 23.2, and 24.9 ppm), an exocyclic methylene group (t at 115.9 ppm), and a carbonyl of the ester type (s at 170.0 ppm). Olefinic absorptions due to C-7, C-8, and C-15 occurred at 135.0, 139.2, and 150.0 ppm respectively. Further, there were absorptions due to two sp^3 carbons bearing oxygen (d at 68.8 and s at 84.7 ppm), seven methylene groups, and two tertiary carbons.

The secondary nature of the alcohol group in cleomeolide (**1**) was confirmed by acetylation with acetic anhydride in pyridine. This gave the crystalline acetate **2**, $C_{22}H_{32}O_4$, in which the proton absorption at δ 4.53 in **1** was shifted to δ 5.83 (br d, J = 10 Hz). Jones oxidation afforded the ketone **4**, $C_{20}H_{28}O_3$, ν_{max} 1710 cm^{-1}. The 1,2-relationship between the OH and lactone functionalities was established as follows. Treatment of **1** with 10% KOH in aqueous methanol followed by acidification with acetic acid and esterification of the product with diazomethane furnished the crystalline epoxide **5**, $C_{21}H_{32}O_3$, whose ^{1}H NMR showed absorption due to the epoxide proton at δ 3.13 (br d; J = 10 Hz). Formation of the epoxide **5** suggested that there was a *trans* relationship between the OH group and the lactone oxygen in cleomeolide. Acid treatment of **5** gave cleomeolide.

Two products were obtained when the ketone **4** was treated with 10% KOH at room temperature: an isomeric lactone, m.p. 190–192°, and the acid **7**. The former could be formulated as the tricyclic lactone **6** on the basis of its spectral properties. Thus, it had no maximum in its UV spectrum above 220 nm, while its IR spectrum showed absorption for OH (3540) and γ-lactone (1763) groups. Its ^{1}H NMR spectrum (Experimental) showed retention of the exocyclic methylene group, no signals for CHOH, but signals for a disubstituted olefin. The structure of **6** was strongly supported by its ^{13}C NMR spectrum (Table 1) and was conclusively established by X-ray crystallographic analysis. A stereoscopic drawing of **6** is shown in Fig. 1, and the pertinent X-ray crystallographic data are given in Table 2. As can be seen from Fig. 1, **6** is a tricarbocyclic compound, formed by a facile transannular reaction between C-3 and C-8 of **4**. The

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Table 1. ^{13}C NMR data for (**1**) and (**6**)[a]

Carbon atom	(1)	(6)
1	41.2 (d)	40.3 (d)
2	b	c
3	69.8 (d)	78.7 (s)
4	84.7 (s)	84.4 (s)
5	b	c
6	36.7 (t)	128.9 (d)
7	135.0 (d)	129.3 (d)
8	139.2 (s)	55.4 (s)
9	b	c
10	b	c
11	42.1 (s)	43.5 (s)
12	39.5 (d)	39.7 (d)
13	b	c
14	b	c
15	150.0 (s)	154.2 (s)
16	24.9 (q)	25.2 (s)
17	170.0 (s)	175.2 (s)
18	23.2 (q)	20.3 (q)
19	15.8 (q)	15.4 (q)
20	115.9 (t)	113.1 (t)

a. Determined in $CDCl_3$ at 25.2 M Hz. Chemical shifts are in p.p.m. downfield from Me_4Si.

b. Unassigned CH_2 absorptions at 23.6, 24.9, 27.2, 29.2, 33.8, and 37.1 p.p.m.

c. Unassigned CH_2 absorptions at 24.8, 26.4, 27.4, 33.5, 33.8, and 36.8 p.p.m.

tricarbocyclic system in **6** is analogous to that found in the taxane[14] group of diterpenes and could be of some biosynthetic significance.

When the lactone **6** was boiled under reflux (1 hr) with 10% KOH in aqueous methanol, followed by acidification, the crystalline hydrocarbon **9**, $C_{19}H_{26}$, was produced. Formation of **9** most likely occurred via the diene **10** in a decarboxylation-elimination process. Indeed, careful neutralization of the reaction mixture led to the detection of an unstable substance whose UV spectrum (λ_{max} 277 nm) was consistent with **10**.

Although the preceding chemical and spectral evidence served to delineate the main structural features of cleomeolide, it was considered insufficient to define unambiguously the stereostructure of this compound. Consequently, an X-ray structure determination of cleomeolide (**1**) was undertaken. A stereoscopic drawing of **1** as determined from the X-ray crystallographic analysis is displayed in Fig. 2. This Figure also represents the absolute stereochemistry as determined from application of Brewster's rule[15] on the benzoate **3**. Details of the X-ray analysis are given in Table 2. Cleomeolide possesses a 12-membered carbocyclic ring *cis* fused to a cyclohexane ring. There is also a 7-membered α,β-unsaturated lactone in which the double bond is at the bridgehead position.[16]

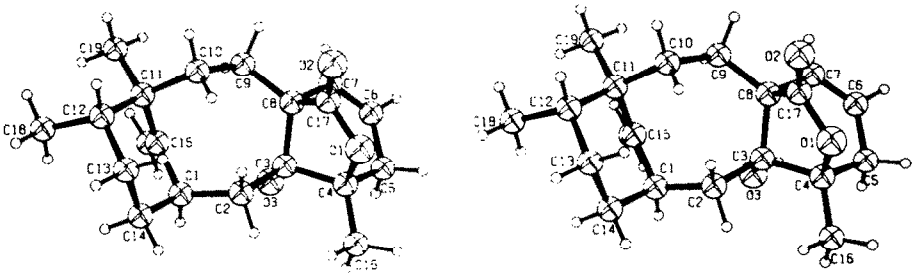
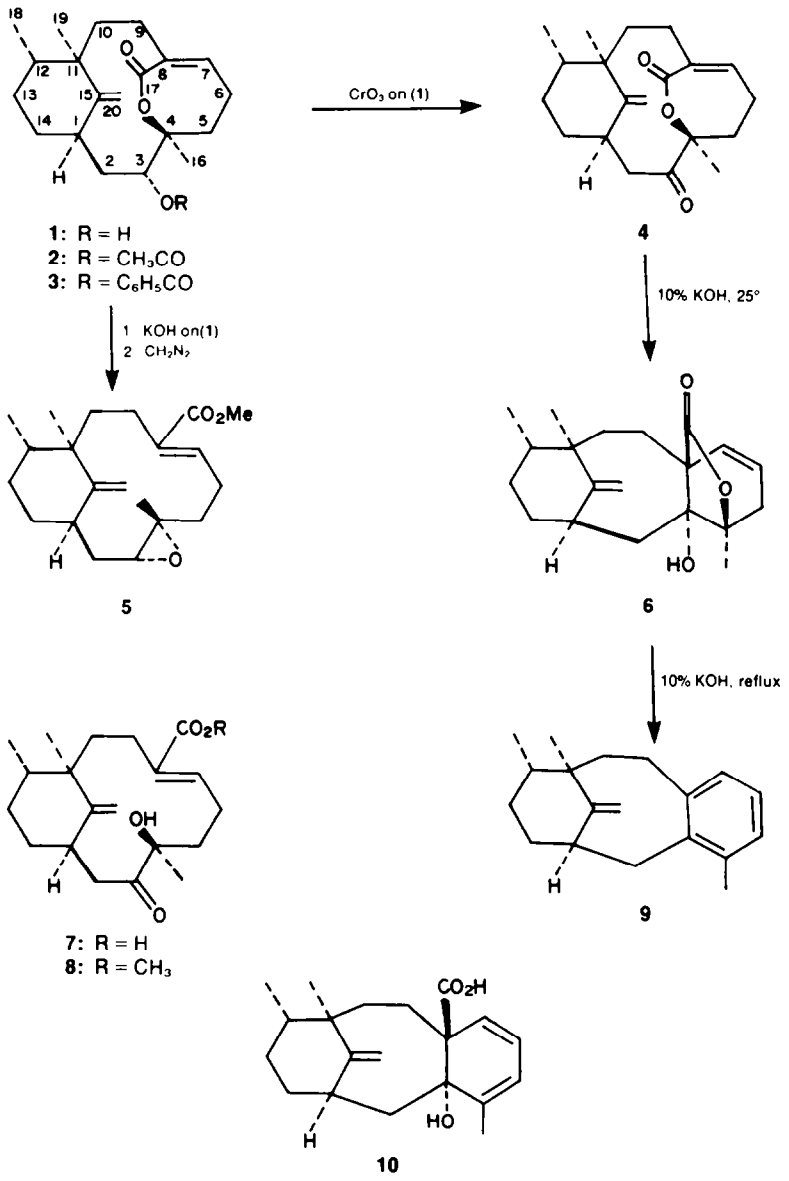


Fig. 1. A stereoscopic ORTEP drawing of lactone (**6**).



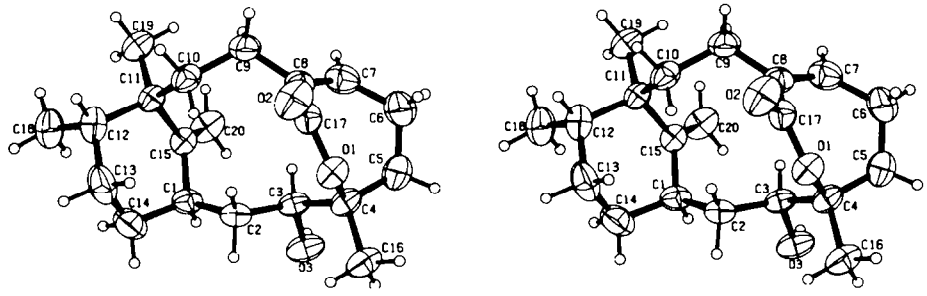
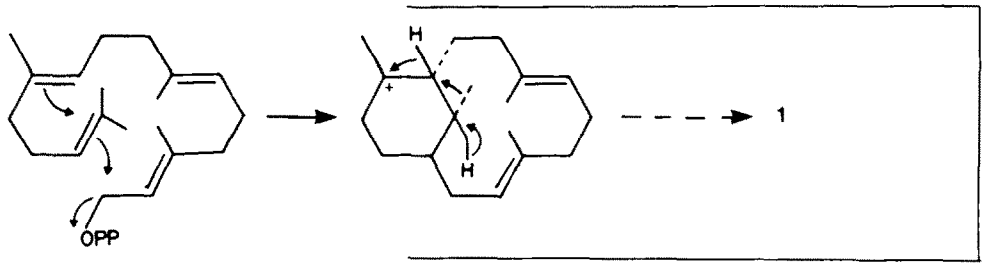


Fig. 2. A stereoscopic ORTEP drawing of cleomeolide (**1**).

Table 2. Crystal data and details of X-ray crystallographic analyses for (1) and (6)

	(1)	(6)
Formula	$C_{20}H_{30}O_3$	$C_{20}H_{28}O_3$
Space group	$P2_1$	$P2_1$
a/Å	10.329 (2)	6.975 (1)
b/Å	12.468 (3)	11.700 (2)
c/Å	7.356 (2)	10.370 (1)
β/°	109.98 (2)	95.64 (1)
Z	2	2
$D_{calcd.}$/gm cm^{-3}	1.187	1.247
μ(Cu-Kα)/cm^{-1}	6.2	6.6
Crystal size/mm	0.15x0.40x0.50	0.20x0.20x0.50
Max. θ/°	57	57
Total reflections	1899	1197
No. of obsd reflections	1859	1186
Final R	0.041	0.034
Final wR	0.051	0.049
Final difference map, largest peak/eÅ^{-3}	< ± 0.3	< ± 0.2

The genesis of the cleomeolide skeleton is readily rationalized in terms of head-to-tail cyclization of geranyl geranyl pyrophosphate followed by hydride shift, Me migration and proton loss, as indicated below.



EXPERIMENTAL

M.p's were determined on a Kofler hot-stage apparatus or in capillaries and are uncorrected. Unless otherwise indicated, UV and IR spectra were determined in EtOH and $CHCl_3$ respectively. NMR spectra were determined in $CDCl_3$ with Me_4Si as an internal standard. Chemical shifts are expressed in parts per million (δ) and coupling constants (J) in Hz (s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet). Mass spectra were recorded using a direct inlet system with an ionization energy of 70 eV; *m/e* values are given with relative intensities in parentheses.

Isolation of cleomeolide (1). Finely ground leaves and twigs of *Cleome viscosa* (3.8 kg), collected near the Mona campus, were extracted by cold percolation with benzene. The green gum (317 g), obtained by removal of the solvent under reduced pressure (below 50°), was chromatographed twice on alumina. Elution with benzene-EtOAc gave fractions which crystallized from MeOH to give *cleomeolide* (1) as prisms, m.p. 216–217°, $[\alpha]_D^{25}$ + 120° ($CHCl_3$, *c* = 1.1); λ_{max} 214 nm (ε 4800); ν_{max} (KBr) 3485, 1683, 1642, 1628, 900 cm^{-1}; δ 0.86 (d, J = 7 Hz, C$\underline{H}_3$-18), 1.00 (s, C$\underline{H}_3$-19), 1.35 (s, C$\underline{H}_3$-16), 4.53 (br d, J = 12 Hz, C$\underline{H}$-3), 4.80 (d, J = 2 Hz, C$\underline{H}_A$-20), 4.90 (d, J = 2 Hz, C$\underline{H}_B$-20), 6.20 (m, C$\underline{H}$-7); *m/e* 318 (M^+, 3), 303 (M^+-CH_3, 2), 300 (M^+-H_2O, 9), 285 (4), 272 (8), 247 (7), 234 (8), 227 (6), 206 (13), 188 (25), 173 (12), 161 (12), 147 (16), 136 (25), 123 (100), 121 (60), 107 (35), 95 (30). (Found: C, 75.6; H, 9.3. $C_{20}H_{30}O_3$ requires: C, 75.4; H, 9.5%).

Cleomeolide acetate (2). This was prepared in the usual manner with Ac_2O and pyridine. It crystallized as needles from MeOH, m.p. 140–143°, $[\alpha]_D^{25}$ + 96° ($CHCl_3$, *c* = 0.89), λ_{max} 216 nm (ε 4800); ν_{max} ($CHCl_3$) 1736, 1700, 900 cm^{-1}; δ 0.83 (d, J = 6.5 Hz, C$\underline{H}_3$-18), 1.01 (s, C$\underline{H}_3$-19), 1.33 (s, C$\underline{H}_3$-16), 4.80 (d, J = 2 Hz, C$\underline{H}_A$-20), 4.93 (d, J = 2 Hz, C$\underline{H}_B$-20), 5.83 (br d, J = 10 Hz, C$\underline{H}$-3), 6.13 (m, C$\underline{H}$-7); *m/e* 360.231. (Found: C, 73.5; H, 9.0. $C_{22}H_{32}O_4$ requires: C, 73.3; H, 8.9%).

Cleomeolide benzoate (3). This was prepared in the usual manner from benzoyl chloride and pyridine at room temp during 16 hr. Purification of the product by preparative scale tlc and crystallization from aqueous MeOH gave the *benzoate* (3), m.p. 157–159°, $[\alpha]_D^{25}$ + 75° ($CHCl_3$, *c* = 1.00), ν_{max} 1710, 1630, 1600, 1580, 920 cm^{-1}; δ 0.83 (d, J = 7 Hz, C$\underline{H}_3$-18), 1.03 (s, C$\underline{H}_3$-19), 1.43 (s, C$\underline{H}_3$-16), 4.97 (d, J = 2 Hz, C$\underline{H}_A$-20), 5.03 (d, J = 2 Hz, C$\underline{H}_B$-20), 6.17 (br d, J = 10 Hz, C$\underline{H}$-3), 6.23 (m, C$\underline{H}$-7), 7.50 (3 H, m, Ar-H), 8.07 (2 H, m, Ar-H). (Found: C, 76.55; H, 8.3. $C_{27}H_{34}O_4$ requires: C, 76.7; H, 8.1%).

Treatment of cleomeolide with alkali. A soln of cleomeolide (250 mg) in 3.4% methanolic KOH (29 mL) was refluxed for

2.5 hr. Most of the MeOH was removed *in vacuo* and, after dilution with H_2O (20 mL), the soln was acidified with HOAc. The product, recovered with EtOAc, was esterified with diazomethane. Evaporation of the solvent gave a gum (2 main components by tlc), which was separated by preparative-scale tlc to give *cleomelide* **1** (41 mg) and the *methyl ester* **5** (210 mg). The latter crystallized from aqueous MeOH as needles, m.p. 78–80°, λ_{max} 218 nm (ε 5950); ν_{max} ($CHCl_3$) 1706, 907 cm^{-1}; δ 0.83 (d, J = 7 Hz, C$\underline{H}_3$-18), 0.90 (s, C$\underline{H}_3$-19), 1.21 (s, C$\underline{H}_3$-16), 3.13 (br d, J = 10 Hz, C$\underline{H}$-3), 3.70 (s, $CO_2C\underline{H}_3$), 4.91 (d, J = 2 Hz, C$\underline{H}_A$-20), 5.08 (d, J = 2 Hz, C$\underline{H}_B$-20), 5.16 (d of d, J = 4, 12 Hz, C$\underline{H}$-7). (Found: C, 75.5; H, 9.6. $C_{21}H_{32}O_3$ requires: C, 75.9; H, 9.7%).

Oxidation of cleomeolide. Cleomeolide **1** (325 mg) in acetone (25 mL) was oxidized with Jones reagent at room temp. Removal of the solvent *in vacuo* followed by dilution with water (50 mL) gave a crystalline ppt of **4** (297 mg), needles from MeOH, m.p. 160–163°, λ_{max} 218 nm (ε 5950); ν_{max} ($CHCl_3$) 1710, 907 cm^{-1}; δ 0.86 (d, J = 7 Hz, C$\underline{H}_3$-18), 1.01 (s, C$\underline{H}_3$-19), 1.45 (s, C$\underline{H}_3$-16), 4.81 (d, J = 2 Hz, C$\underline{H}_A$-20), 5.00 (d, J = 2 Hz, C$\underline{H}_B$-20), 5.98 (m, C$\underline{H}$-7). (Found: C, 75.7; H, 8.8. $C_{20}H_{28}O_3$ requires: C, 75.9; H, 8.9%).

Base treatment of ketone (**4**). A soln of **4** (193 mg) in 10% KOH in MeOH (6.8 mL) was stirred at room temp for 4 hr. Most of the solvent was removed *in vacuo* and the residue diluted with H_2O (25 mL). The ppt was filtered off, washed well with H_2O, dried and recrystallized from MeOH to furnish the *tricyclic lactone* (**6**) as needles (75 mg), m.p. 190–192°, ν_{max} (KBr) 3540, 1763, 1638, 887 cm^{-1}; δ 0.88 (d, J = 7 Hz, C$\underline{H}_3$-18), 0.94 (s, C$\underline{H}_3$-19), 1.34 (s, C$\underline{H}_3$-16), 4.74 (d, J = 2 Hz, C$\underline{H}_A$-20), 5.16 (d, J = 2 Hz, C$\underline{H}_B$-20), 5.18 (d of t, J = 1, 9 Hz, C$\underline{H}$-7), 5.86 (d of t, J = 4, 9 Hz, C$\underline{H}$-6); MS: *m/e* 316 (M^+, 5), 301 (5), 272 (10), 257 (7), 207 (25), 163 (45), 123 (56), 43 (100). (Found: C, 76.0; H, 8.9. $C_{20}H_{28}O_3$ requires: C, 75.9; H, 8.9%).

The filtrate from the preceding experiment was acidified with dil HCl and the resulting mixture was extracted with EtOAc (4 × 30 mL). The combined organic layers were washed with H_2O (2 × 20 mL) and evaporated to **7**, m.p. 98–100° (from aq. MeOH), which was characterized as its *methyl ester* (**8**) by treatment with CH_2N_2. It crystallized from aq. MeOH (needles), m.p. 126–127°, λ_{max} 224 nm (ε 5200); ν_{max} 3460, 1718, 1706 cm^{-1}; δ 0.83 (s, C$\underline{H}_3$-18), 0.86 (d, J = 6.5 Hz, C$\underline{H}_3$-19), 1.16 (s, C$\underline{H}_3$-16), 3.80 (s, $CO_2C\underline{H}_3$), 4.81 (d, J = 2 Hz, C$\underline{H}_A$-20), 5.52 (d, J = 2 Hz, C$\underline{H}_B$-20), 5.53 (m, C$\underline{H}$-7). (Found: C, 72.35; H, 9.3. $C_{21}H_{32}O_4$ requires: C, 72.4; H, 9.3%).

The aromatic hydrocarbon (**9**). A soln of **6** (55 mg) and 10% KOH in MeOH was boiled under reflux for 1 hr. After removal of the solvent *in vacuo*, the mixture was diluted with H_2O (8 mL) and filtered. The filtrate was acidified with dil HCl and extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with H_2O (2 × 10 mL) and evaporated. The product **9** was then recrystallized from aqueous MeOH to give plates (20 mg), m.p. 87–89°, ν_{max} 1631, 898 cm^{-1}; δ 0.86 (d, J = 7 Hz, C$\underline{H}_3$-18), 0.91 (s, C$\underline{H}_3$-19), 2.30 (s, C$\underline{H}_3$-16), 4.13 (d, J = 2 Hz, C$\underline{H}_A$-20), 4.45 (d, J = 2 Hz, C$\underline{H}_B$-20), 6.93 (m, C$\underline{H}$-5, C$\underline{H}$-6, C$\underline{H}$-7); *m/e* 254 (M^+, 78), 249 (8), 226 (54), 212 (14), 197 (12), 184 (15), 171 (35), 156 (63), 143 (52), 131 (38), 123 (100), 118 (45), 107 (50), 91 (40), 81 (42) (M^+ 254.2049; $C_{19}H_{26}$ requires: 254.2036).

X-Ray crystallography. The crystallographic data for **1** and **6** were collected on a fully-automated Hilger-Watts diffractomer (Cu–Kα radiation, θ 2θ scans, pulse height discriminations, no absorption corrections applied), and are summarized in Table 2. The structure and relative stereochemistry of **1** and **6**, using crystals grown from MeOH, were solved by a multiple soln procedure[17] and refined by full matrix least-squares. In the final refinement, anisotropic thermal parameters were used for non-H atoms and isotropic factors for the H atoms. H atoms were included in the structure factor calculation but their parameters were not refined. Listings of final atomic parameters, anisotropic thermal parameters, bond lengths, bond angles, torsion angles, and observed and calculated structure factors (22 p.p.) are submitted as supplementary material to be deposited in the Cambridge University Crystallographic Centre.[18]

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[16] During the preparation of our manuscript a paper appeared in which the X-ray structure of **1** was disclosed (S. B. Mahato, B. C. Pal, T. Kawasaki, K. Miyahara, O. Tanaka and K. Yamasaki, *J. Am. Chem. Soc.* **101,** 4720 (1979).

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