Structure of Cleomeolide, a Novel Diterpene Lactone from *Cleome icosandra* Linn.

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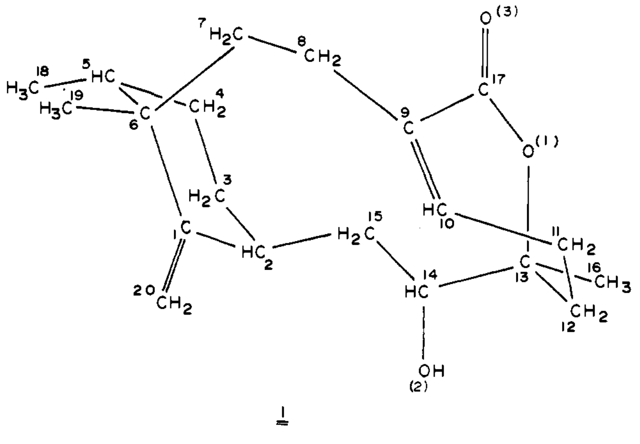
Abstract: The structure and stereochemistry of cleomeolide (**1**), a novel diterpene lactone isolated from *Cleome icosandra* Linn. (Syn. *Cleome viscosa* Linn.), have been determined by means of X-ray analysis and NMR and CD spectra. The compound, $C_{20}H_{30}O_3$, is monoclinic, with space group $P2_1$ and unit cell dimensions of a = 10.303 (4) Å, b = 12.436 (4) Å, c = 7.341 (3) Å, β = 110.02 (3)°, V = 883.70 Å^3, and Z = 2. The crystal structure was solved by the direct method and refined by the routine least-squares method including anisotropic (for nonhydrogen atoms) and isotropic (for hydrogen atoms) thermal parameters to a final R value of 0.052. The absolute configuration was determined from the CD curve of the ketone of **1**. Cleomeolide possesses a 12-membered carbocyclic ring fused to a cyclohexane ring. It also contains a seven-membered α,β-unsaturated lactone ring.

Cleome icosandra Linn. (Syn. *Cleome viscosa* Linn.) (Capparidaceae) is a sticky herb with yellow flowers and strong penetrating odor. It is widely distributed in India and has a reputation for its rubefacient, vesicant and anthelmintic properties.[2] Previous studies[3,4] on this plant had disclosed the presence of a flavone and some unsaturated fatty acids. We have now isolated from this plant a diterpene lactone, cleomeolide (**1**), having a new skeleton.

Results and Discussion

Cleomeolide, mp 224–225 °C, $[\alpha]^{27}_{D}$ +100° ($CHCl_3$), was isolated by adsorption chromatography from the petroleum ether extract of the aerial part of the plant. Molecular weight determination by mass spectral and elemental analysis established the molecular formula $C_{20}H_{30}O_3$ for **1**. The compound was soluble in alkali. Its UV spectrum showed absorption at 225 nm (ϵ 3498). The IR spectrum showed a strong band at 1700 cm^{-1}. These data indicated that cleomeolide might contain a seven-membered α,β-unsaturated lactone ring. The other bands discernible in the IR spectrum were at 3400 (hydroxyl), 1650, and 890 cm^{-1} (exocyclic methylene). The ^1H NMR spectrum contained signals for two quaternary methyl groups, one secondary methyl group, one exocyclic methylene, a trisubstituted double bond and a carbinyl proton. The presence of the secondary hydroxyl group in **1** was also established by preparation of its acetate, *p*-bromobenzoate, and ketone.



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The nature of the 20 carbon atoms was revealed from ^{13}C NMR studies using techniques, i.e., proton-noise decoupling, continuous wave decoupling, and partially relaxed Fourier transform (PRFT).[5] These analyses indicate that cleomeolide has one lactone (-COOC<), one secondary hydroxyl (>CHOH), two double bonds (>C=CH- and >C=CH$_2$), three methyls, seven methylenes, two methines, and only one quaternary carbon. The ^{13}C NMR data are shown in Table I. Assignments of the pertinent signals are made on the basis of their chemical shifts.[6]

The detailed structure and stereochemistry of cleomeolide were established unambiguously by single-crystal X-ray diffraction. A stereoscopic view[7] of the molecule is shown in Figures 1 (including H atoms) and 2 (no H atoms are given). Bond angles are given in Figure 3 and Figure 4 shows the bond distances. Cleomeolide possesses a 12-membered carbocyclic ring fused to a cyclohexane ring. It also contains a seven-membered α,β-unsaturated lactone ring. This represents the first member of a hitherto unknown skeletal system.[8]

The conformational stability of the molecule of cleomeolide in solution was revealed from a study of its ^1H NMR spectrum and that of its acetate. In the case of cleomeolide the carbinyl proton was observed at δ 4.54 as a broadened doublet (J = 11 and 3.5 Hz). This signal was shifted to δ 5.9 in case of the acetate and was observed as a clean doublet (J = 11 Hz). Thus the smaller coupling constant in the alcohol is presumably due to coupling with the hydroxyl proton. The above splitting pattern is readily explained by assuming that the conformation of the molecule in solution is the same as that of crystalline **1**, which shows that the dihedral angles for the carbinyl proton with the two vicinal methylene protons are 88.6 and 161.3° (vide torsion angles[9]).

The CD curve of **1** shows a strong positive Cotton effect ($[\theta]_{235}^{EtOH}$ +8612) due to the α,β-unsaturated lactone chromophore and the CD of the ketone shows $[\theta]_{243}^{CHCl_3}$ +7932 due to the lactone and $[\theta]_{300}^{CHCl_3}$ +610 for the ketone chromophore. Although the Klyne lactone sector rule[10] or the Snatzke lactone rule[11] could not be applied with confidence to this type of α,β-unsaturated lactone for determination of absolute configuration, the small positive Cotton effect shown by the ketone was useful for the purpose. An examination of the molecular model of the ketone shows that, if the absolute configuration is similar to that of the alcohol depicted as **1** (which is the antipode of Figure 1), application of the octant rule[12] predicts a positive Cotton effect. Almost the whole of the cyclohexane ring (upper left) and the methyl group α to the carbonyl (lower right) contribute to the positive Cotton

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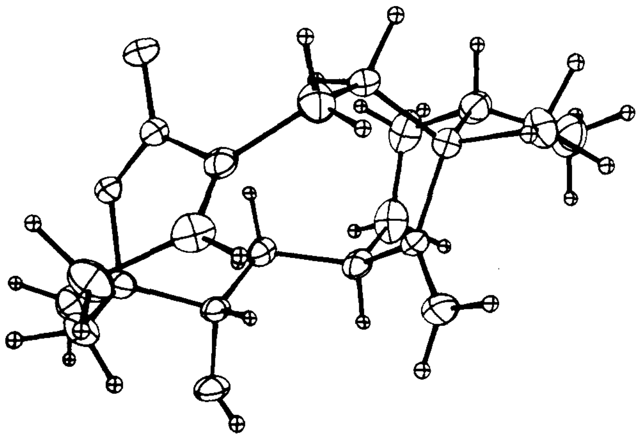


Figure 1. Stereoscopic view of single molecule (including H atoms).

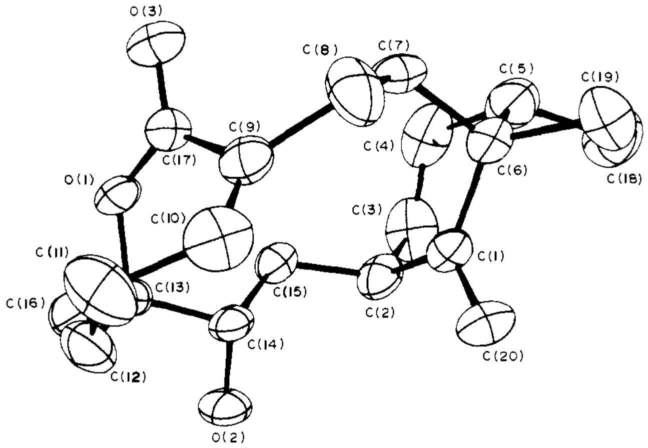


Figure 2. Stereoscopic view of single molecule (no H atoms are given).

Table I. ^{13}C NMR Shifts of **1** (ppm) (Me_4Si = 0), $CDCl_3$

δ	multiplicity	assignment
15.8	q	C-18
23.2	q	C-19
24.9	q	C-16
23.6	t	C-3, C-4, C-7, C-8, C-11, C-12, and C-15
24.9	t	
27.2	t	
29.3	t	
33.9	t	
36.8	t	
37.1	t	
39.5	d	C-2 and C-5
41.3	d	
42.1	s	C-6
69.9	d	C-14
84.8	s	C-13
116.1	t	C-20
135.2	d	C-10
139.4	s	C-9
150.2	s	C-1
170.3	s	C-17

effect which is partially compensated by the contribution of a part of the lactone ring (upper right).

The biogenetic pathway of formation of cleomeolide may be envisaged as occurring via oxidative cyclization of geranyl linalool epoxide as outlined in Scheme I. We are searching for compounds related to cleomeolide which might provide some insight into this question.

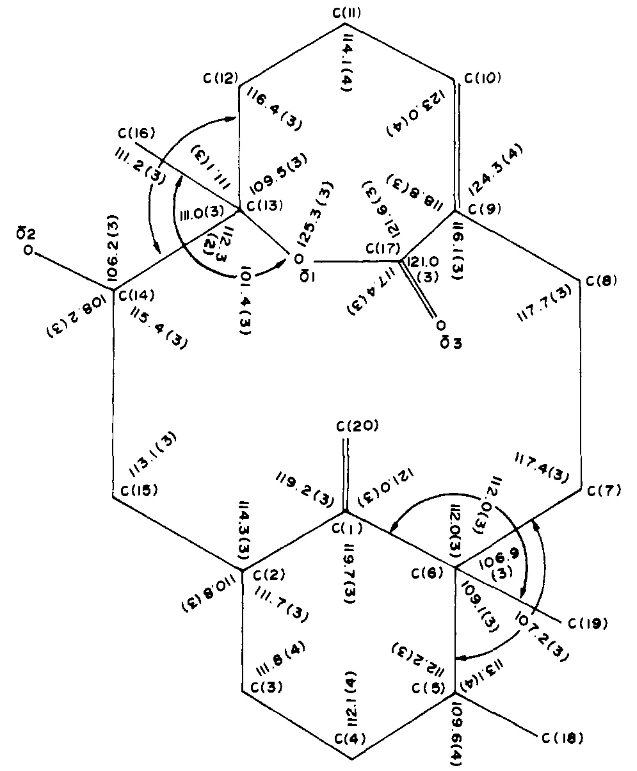


Figure 3. Bond angles with standard deviations for last digit in parentheses.

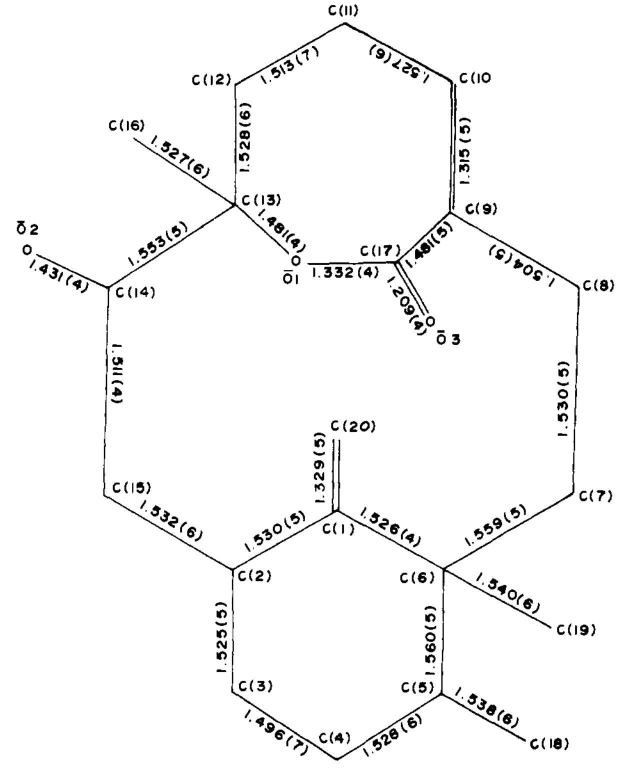


Figure 4. Bond distances with standard deviations for last digit in parentheses.

Experimental Section

Melting points are uncorrected. UV spectra were recorded on a Specord UV-vis instrument, IR spectra were taken on a Perkin-Elmer Infracord Model 137, and ^{1}H NMR spectra were taken in $CDCl_3$ solution on a JEOL-PFT-100 spectrometer at 100 MHz with Me_4Si as internal reference. The ^{13}C NMR spectra were taken in $CDCl_3$ solution (0.3 M) on a JEOL-PFT-100 spectrometer (25.15 MHz)

Table II. Final Atomic Parameters and Anisotropic Thermal Parameters for Nonhydrogen Atoms[a]

atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}	Beqv
C(1)	6883(3)	−1036(3)	2087(4)	39(1)	44(2)	35(1)	−5(1)	18(1)	−3(1)	3.0
C(2)	6384(4)	−2202(3)	1723(5)	52(2)	42(2)	49(2)	−6(2)	28(2)	−11(1)	3.5
C(3)	7490(4)	−2991(3)	2883(7)	62(3)	43(2)	104(4)	8(2)	37(2)	−2(2)	5.3
C(4)	8102(4)	−2669(4)	4968(7)	54(2)	64(3)	91(3)	24(2)	32(2)	36(2)	5.3
C(5)	8756(4)	−1552(4)	5207(5)	40(2)	80(3)	45(2)	6(2)	13(1)	11(2)	4.4
C(6)	7695(3)	−666(3)	4146(4)	36(1)	48(2)	37(1)	−5(1)	14(1)	−1(1)	3.2
C(7)	6738(3)	−474(3)	5364(5)	43(2)	63(2)	37(1)	−7(2)	17(1)	−13(2)	3.7
C(8)	5626(4)	393(3)	4650(6)	53(2)	49(2)	73(2)	−9(2)	26(2)	−28(2)	4.5
C(9)	4176(3)	31(3)	3540(5)	48(2)	44(2)	41(2)	−2(1)	21(1)	−10(1)	3.4
C(10)	3325(4)	543(3)	2039(6)	71(2)	41(2)	58(2)	3(2)	23(2)	3(2)	4.5
C(11)	1801(4)	248(4)	1122(6)	64(2)	64(3)	67(3)	17(2)	4(2)	11(2)	5.5
C(12)	1546(4)	−841(4)	146(5)	46(2)	81(3)	48(2)	3(2)	5(2)	5(2)	4.9
C(13)	2496(3)	−1752(3)	1214(4)	45(2)	54(2)	33(1)	−11(2)	10(1)	−4(1)	3.5
C(14)	3814(3)	−1808(3)	646(4)	49(2)	42(2)	30(1)	−12(1)	16(1)	−6(1)	3.1
C(15)	5011(3)	−2419(3)	2037(4)	48(2)	34(1)	41(1)	−6(1)	19(1)	−1(1)	3.1
C(16)	1735(4)	−2828(4)	853(6)	58(2)	60(2)	62(2)	−26(2)	24(2)	−16(2)	4.7
C(17)	3632(3)	−857(3)	4416(4)	37(1)	59(2)	35(1)	3(2)	14(1)	−1(2)	3.4
C(18)	10035(4)	−1578(4)	4580(7)	41(2)	89(3)	82(3)	9(2)	26(2)	4(3)	5.5
C(19)	8471(4)	395(3)	4166(6)	48(2)	58(2)	61(2)	−19(2)	14(2)	−7(2)	4.5
C(20)	6610(4)	−364(4)	597(5)	64(2)	73(3)	39(2)	−19(2)	15(2)	8(2)	4.7
O(1)	2826(2)	−1622(2)	3330(3)	47(1)	55(1)	34(1)	−7(1)	18(1)	3(1)	3.5
O(2)	3414(3)	−2334(2)	−1198(3)	78(2)	63(2)	34(1)	−21(2)	21(1)	−13(1)	4.6
O(3)	3895(3)	−909(3)	6149(3)	64(2)	99(2)	31(1)	−11(2)	19(1)	−2(1)	5.1

[a] The x, y, and z (all $\times 10^4$) are atomic coordinates. The anisotropic parameters (all $\times 10^3$) are used in the expression $\exp(-[2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}hla^*c^* + 2U_{23}klb^*c^*)])$. Least-squares standard deviations in the final digit are in parentheses.

Table III. Final Atomic Parameters ($\times 10^3$) and Isotropic Thermal Parameters for Hydrogen Atoms[a]

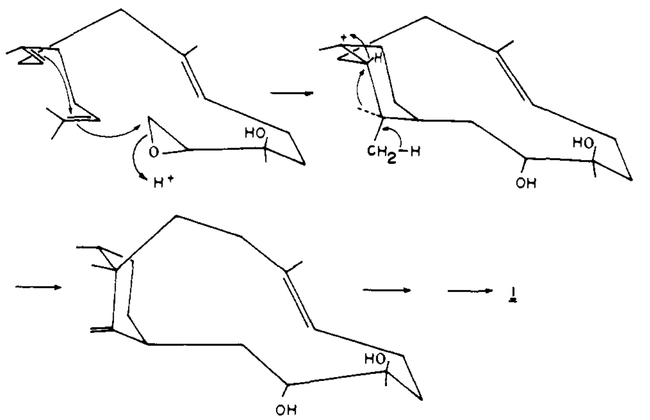
atom	x	y	z	B
H2	621(4)	−249(3)	35(5)	3.0(0.9)
H3(1)	827(4)	−314(3)	225(6)	3.1(1.0)
H3(2)	708(4)	−373(4)	265(6)	3.3(1.0)
H4(1)	876(4)	−318(3)	563(5)	2.6(0.9)
H4(2)	752(4)	−270(3)	560(5)	2.3(0.9)
H5	903(4)	−142(3)	651(5)	2.6(0.9)
H7(2)	634(4)	−111(3)	565(5)	2.2(0.8)
H7(1)	737(4)	−18(3)	665(6)	3.0(0.9)
H8(1)	584(4)	100(3)	397(6)	3.0(0.9)
H8(2)	561(4)	81(3)	572(5)	2.9(0.9)
H10	367(4)	119(3)	154(5)	2.6(0.9)
H11(1)	133(4)	87(3)	11(6)	3.1(1.0)
H11(2)	131(4)	35(4)	223(5)	3.3(0.9)
H12(1)	173(4)	−58(4)	−103(6)	3.6(1.0)
H12(2)	54(4)	−99(4)	−9(6)	3.5(1.0)
H14	410(3)	−106(3)	45(5)	1.7(0.8)
H15(1)	479(4)	−320(3)	186(5)	2.3(0.9)
H15(2)	515(4)	−237(3)	339(5)	2.1(0.8)
H16(1)	240(4)	−346(3)	137(6)	2.8(0.9)
H16(2)	94(4)	−285(3)	129(5)	2.8(0.9)
H16(3)	136(4)	−303(3)	−52(6)	2.9(0.9)
H18(1)	1061(4)	−95(4)	501(5)	3.2(0.9)
H18(2)	1057(4)	−220(4)	510(6)	3.6(1.0)
H18(3)	982(4)	−169(4)	320(5)	3.2(0.9)
H19(1)	788(4)	104(3)	382(5)	2.5(0.9)
H19(2)	913(4)	56(3)	542(5)	2.8(0.9)
H19(3)	917(4)	44(3)	344(5)	2.5(0.9)
H20(1)	613(4)	−51(3)	−69(5)	3.0(0.9)
H20(2)	692(4)	49(3)	67(5)	3.0(0.9)
OH2	348(4)	−198(3)	−194(5)	2.5(0.9)

[a] Standard deviations for last digit are in parentheses.

under the following conditions: temperature 26 °C; pulse width 25 μs (90°); repetition time 2 s; accumulation time 2000; spectral width 5 and 6.25 kHz; acquisition time 0.4 and 0.3 s, respectively; data points 4096. A 10-mm φ sample tube was used. The chemical shifts are expressed as δ (ppm) from internal Me_4Si. Mass spectra were obtained on a Hitachi RMU-6L spectrometer at an ionizing potential of 70 eV.

Isolation of Cleomeolide (1). The aerial part of *C. icosandra* Linn. (2 kg) was air dried, powdered, and then extracted exhaustively with petroleum ether in a Soxhlet extractor for 18 h. The dark brown residue (40 g) obtained after removal of the solvent was chromatographed on a column of silica gel (1 kg) using petroleum ether, petroleum ether-benzene mixture (1:1), benzene, benzene-chloroform mixture (1:1), and chloroform as successive eluents. The chloroform eluate (2.5 g) was rechromatographed on silica gel (50 g) using chloroform to give 130 mg of a white solid which on crystallization from MeOH

Scheme I



afforded 80 mg of cleomeolide (**1**) in needles: mp 224–225 °C; $[\alpha]_D{}^{27}$ +100° (c 0.51, $CHCl_3$); UV (EtOH) 225 nm (ϵ 3498); IR (Nujol) 3400 (OH), 1700 (seven-membered α,β-unsaturated lactone carbonyl), 1650 and 890 (exocyclic methylene), 1410, 1272, 1220, 1200, 1050, 1022, 920, 900, 862, and 840 cm^{-1}; ^1H NMR δ 0.80 (d, J = 7 Hz, 18-Me), 0.99 (s, 19-Me), 1.18 (s, 16-Me), 4.54 (br d, J = 11 and 3.5 Hz, 14-H), 4.86 (d, J = 2 Hz, 20-H), 4.94 (d, J = 2 Hz, 20-H), and 6.0 (m, 10-H); MS m/e (%) 318 (3) (M^+), 300 (9) ($M^+ - H_2O$), 285 (3) ($M^+ - H_2O - CH_3$), 272 (8) ($M^+ - H_2O - CO$), 257 (5) ($M^+ - H_2O - CO - CH_3$), 247 (8), 219 (7), 206 (15), 205 (14), 188 (25), 177 (9), 173 (11), 161 (15), 159 (9), 147 (15), 145 (11), 136 (29), 123 (100), 121 (67), 109 (31), 107 (34), 105 (27), 95 (40), 93 (36), 91 (34), 81 (46). Anal. Calcd for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50. Found: C, 75.40; H, 9.48.

Cleomeolide Acetate. A solution of 25 mg of **1** in 2 mL of pyridine and 3 mL of acetic anhydride was kept for 1 h on steam bath. Standard workup yielded the acetate which crystallized from MeOH as microneedles (25 mg): mp 138–140 °C; $[\alpha]_D{}^{27}$ + 70.9° (c 0.82, $CHCl_3$); UV (EtOH) 225 nm (ϵ 3589); IR (Nujol) 1735, 1240 (acetate), 1700 (lactone carbonyl), 1650, 885 (exocyclic methylene), 1220, 1122, 1080, 1045, 1020, 962, 900, 880, and 840 cm^{-1}; ^1H NMR δ 0.82 (d, J = 7 Hz, 18-Me), 1.00 (s, 19-Me), 1.32 (s, 16-Me), 2.08 (s, acetoxy methyl), 4.88 (br s, 20-H), 5.0 (br s, 20-H′), 5.9 (d, J = 11 Hz, 14-H), and 6.2 (m, 10-H); MS m/e (%) 360 (8) (M^+), 332 (7) ($M^+ - CO$), 300 (13) ($M^+ - AcOH$), 285 (6) ($M^+ - AcOH - CH_3$), 272 (14) ($M^+ - AcOH - CO$), 205 (10), 199 (11), 188 (27), 173 (29), 159 (24), 147 (29), 136 (100), 123 (78), 121 (91), 107 (63), 105 (59), 95 (57), 93 (67), 91 (66), 81 (70). Anal. Calcd for $C_{22}H_{32}O_4$: C, 73.30; H, 8.95. Found: C, 73.29; H, 8.85.

Ketone of 1. To a solution of 35 mg of **1** in benzene (25 mL) was added chromium trioxide (80 mg) in glacial acetic acid (5 mL) and water (2.5 mL) and the solution was shaken vigorously for 5 h. Usual workup yielded the ketone, which crystallized from MeOH in needles (24 mg): mp 166–168 °C; UV (EtOH) 224.5 nm (ϵ 3376); IR (Nujol) 1710 (ketone), 1700 (lactone carbonyl), 1645 and 890 (exocyclic methylene), 1220, 1050, 1020, 918, 900, 860, 840 cm^{-1}; ^1H NMR δ 0.84 (d, J = 7 Hz, 18-Me), 1.00 (s, 19-Me), 1.44 (s, 16-Me), 3.0–3.8 (m, 15-H_2), 4.82 (br s, 20-H), 5.06 (br s, 20-H′), and 6.04 (m, 10-H); MS m/e (%) 316 (6) (M^+), 288 (51) ($M^+ - CO$), 273 (35) ($M^+ - CO - CH_3$), 260 (28) ($M^+ - 2CO$), 245 (17) ($M^+ - 2CO - CH_3$), 231 (34), 217 (70), 215 (78), 205 (56), 188 (53), 175 (29), 173 (34), 161 (41), 159 (27), 147 (45), 145 (30), 136 (30), 135 (48), 133 (46), 123 (68), 121 (100), 119 (57), 109 (54), 107 (81), 105 (69), 95 (81), 93 (88), 91 (78), and 81 (86). Anal. Calcd for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.89; H, 8.93.

***p*-Bromobenzoate of 1.** The derivative was prepared in the usual way by using 30 mg of **1**, 80 mg of *p*-bromobenzoyl chloride, and 1 mL of pyridine. Standard workup yielded the *p*-bromobenzoate of **1**, which crystallized from MeOH as prisms: mp 171–172 °C; MS m/e (%) 502 (43), 500 (42), 407 (4), 317 (8), 300 (46), 272 (32), 255 (23), 241 (18), 220 (18), 213 (14), 206 (27), 188 (28), 185 (100), 183 (100), 173 (25), 157 (24), 155 (24), 147 (23), 145 (22), 136 (77), 133 (29), 123 (44), 121 (43), 107 (29), 105 (32), 95 (20), 93 (20), and 91 (25).

Crystallization and Crystal Data. A crystal suitable for X-ray analysis was obtained from 1,2-dichloroethane solution as a colorless block with approximate dimensions of 0.25 × 0.3 × 0.5 mm which has the following data: molecular formula $C_{20}H_{30}O_3$, crystal system monoclinic, space group $P2_1$ (indicated by systematic extinctions, $0k0$ with k odd), a = 10.303 (4) Å, b = 12.436 (4) Å, c = 7.341 (3) Å, β = 110.02 (3)°, d(calcd) = 1.197 g/cm^3, d(obsd by flotation method in an aqueous solution of potassium iodide) 1.182 g/cm^3 (24 °C), Z = 2, V = 883.70 $Å^3$.

The three-dimensional intensities for the crystal were collected on a Syntex $P\bar{1}$ fully automated four-circle diffractometer by the θ–2θ scanning technique with graphite monochromated Mo Kα radiation (0.710 69 Å). Measurement was performed for 3564 independent reflections with $2\theta \leq 65°$, among which 2299 (65%) were regarded to be observed ($I_0 \geq 1.6\sigma$). All the intensities were corrected for Lorentz and polarization factors and the reflections, including unobserved ones, were placed on an approximate absolute scale by Wilson plot.

Structure Determination. The structure was solved by the direct method using 205 reflections with E = 1.70.[13] An E synthesis for a phase set having the lowest R value revealed 18 plausible nonhydrogen atoms. The remaining five atoms were found on difference synthesis based on the known coordinates. The block-diagonal least-squares refinements for 2299 reflections with isotropic temperature factors for hydrogen atoms and anisotropic temperature factors for the remaining atoms converged to the final R factor of 0.052.

All the calculations were performed on a FACOM M-190 computer operated by the Computer Center of Kyushu University by using the UNICS II[14] and the MULTAN programs.[13]

Table II lists nonhydrogen atomic coordinates and thermal parameters and Table III shows hydrogen atomic parameters.

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Supplementary Material Available: Listings of the torsion angles and structure factor amplitudes (18 pages). Ordering information is given on any current masthead page.

References and Notes

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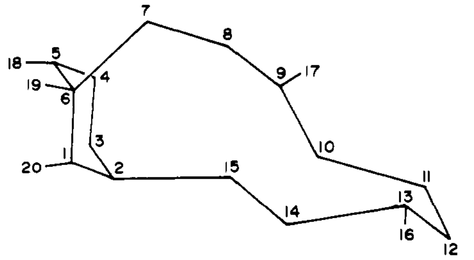
(8) We recommend the name cleomane for the new carbon skeleton present in cleomeolide and suggest the numbering as shown in **2**.



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