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A NEW DITERPENE FROM *CUPRESSUS GOVENIANA* VAR. *ABRAMASIANA*: 5β-HYDROXY-6-OXASUGIOL (CUPRESOL)

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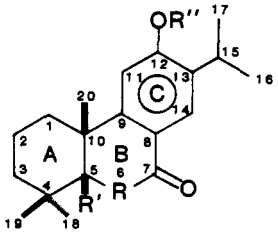
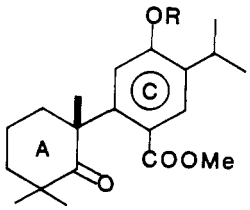
ABSTRACT.—The petroleum ether-EtOH extract of *Cupressus goveniana* var. *abramasiana* (Cupressaceae) yielded sugiol (**1**) and the new diterpene, cupresol (5β-hydroxy-6-oxasugiol), for which structure **2** was established by spectroscopic and chemical means.

We have isolated two diterpenes from the tumor-inhibitory fraction of the petroleum ether-EtOH extract of the stem bark of *Cupressus goveniana* var. *abramasiana* (C.B. Wolf) Little (Cupressaceae). The major one was sugiol (**1**), which has been previously isolated from numerous sources and characterized (1-10). The minor one, apparently a biosynthetic oxidation product of sugiol (**1**), with the lactol structure **2**, is closely related to lactol (**3**) from the bark of *Podocarpus ferrugineus* (11,12). The latter, whose ^{1}H-nmr parameters are included in Table 1, was obtained by Wenkert *et al.* from the oxidation of sugiyl methyl ether with molecular oxygen in KO-*t*-Bu-*t*-BuOH (11,12).





	R	R′	R″
1	CH_2	α-H	H
1a	CH_2	α-H	Ac
2	O	β-OH	H
3	O	β-OH	Me

	R
4	H
5	Me

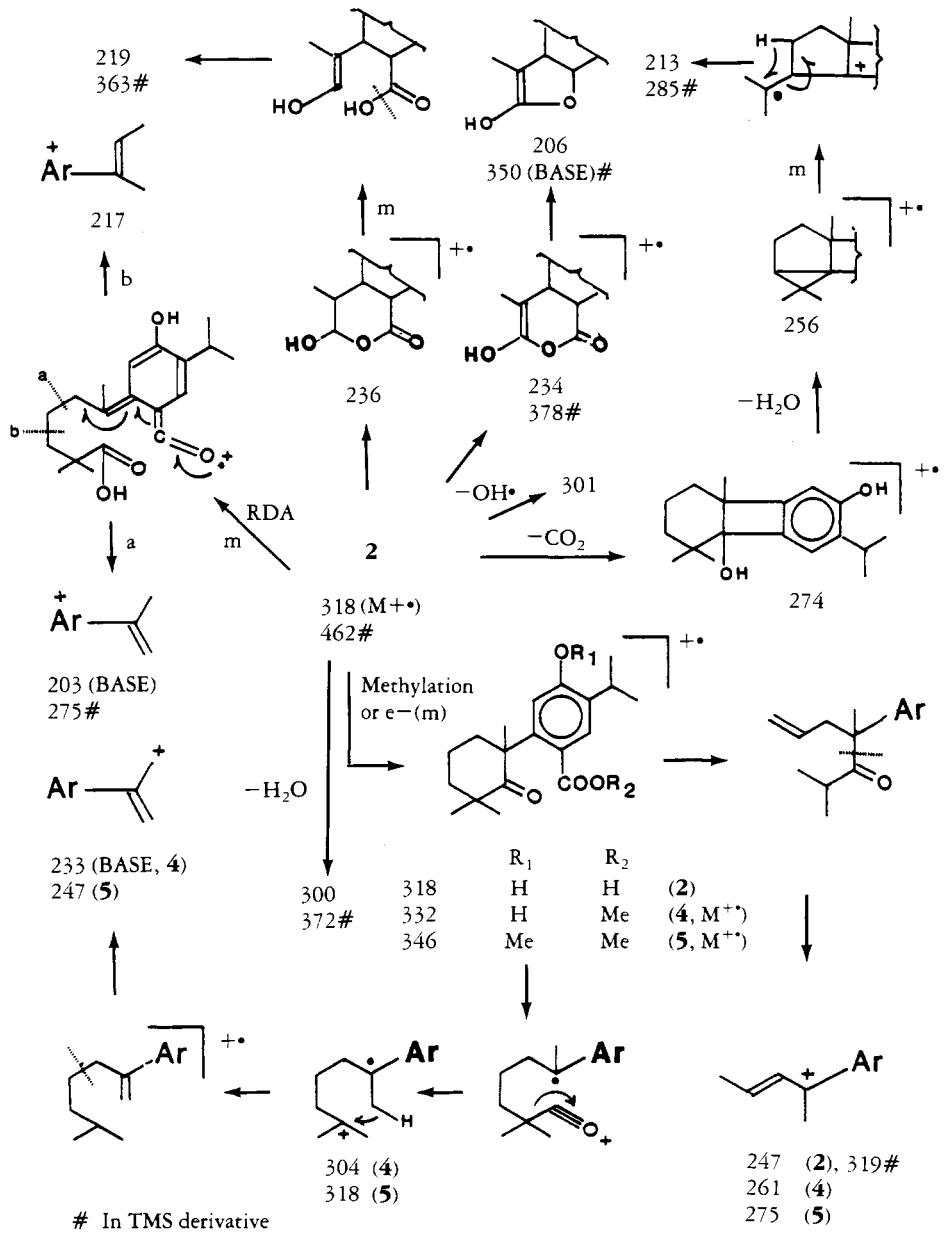
The evidence for structure **2** follows. Hrms indicated a molecular formula $C_{19}H_{26}O_4$. The ir spectrum showed absorptions for conjugated carbonyl and tertiary hydroxyl groupings. The ^{1}H-nmr spectral parameters (Table 1) of **2** are similar to those of **3** with the major difference being that the methoxyl absorption of **3** is replaced by hydroxyl absorption in **2**. As noted in Table 1, the 1α, 18-Me, and δ 1.34 Me signals are broadened but sharpen on warming the sample; this is apparently due to the presence of appreciable amounts of both chair forms of ring A with an appreciable barrier between them. In the chair form with the aromatic ring and 18-Me axial, the latter lies over the former and is strongly shielded; this requires a *cis*-A/B ring juncture as shown (11,12). The 1α proton is strongly deshielded in this conformation. The broadening is greatest for the protons whose shifts are most different between the two chair forms. Similar broadenings are observed in the cmr spectrum (Table 2) which contains the signals expected of structure **2**.

TABLE 1. ^{1}H-nmr Chemical Shifts (δ) and Coupling Constants (Hz, in parentheses) for **1-5**

H	**1**[a]	**2**[b]	**3**[b] (8)	**4**[b]	**5**[b]
1α	~1.32 m	2.06 bd (14)[d]		2.31 td (13.1, 3.5)	2.39 (13.0, 3.9)
1β	1.98 dt (11.4, 2.7)	~1.74 m		~1.60 m	~1.60 m
2α	~1.32 m			1.69 df (13.4, 3.5)	1.71 (13.4, 3.5)
2β	1.49 qt (13.5, 2.7)			1.96 (13.4, 3.1)	1.98 (13.1, 3.3)
3α	1.01 td (13.1, 3.5)	1.4-1.7 m		2.28 td (13.1, 3.5)	2.30 (13.3, 3.6)
3β	~1.32 m			~1.60	~1.60 m
5	1.67 dd (12.9, 5.0)	—		—	—
6α	2.65 dd (18.0, 5.0)	—		—	—
6β	2.58 dd (18.0, 12.9)	—		—	—
11	6.99 d (0.7)	6.78 s	6.80	6.83	6.98
14	8.27 d (0.7)	7.94 s	7.90	7.79	7.80
15	3.50 heptet (7.0)	3.18 (7.0)	3.2 (7)	3.03 (6.9)	3.25 (6.9)
16,17	1.25 d (7.0), 1.26 d (7.0)	1.26 (6.9), 1.27 (6.9)	1.22 (7)	1.17 (6.9)	1.19 (6.9), 1.21 (6.9)
18	0.71 s	0.69 (bs)[c]	0.70	1.28, 1.30	1.24, 1.29
19	0.76 s	1.14	1.19		
20	1.04 s	1.34 bs[d]	1.39	1.60	1.65
CO_2Me	—	—		3.79 s	3.79
ArOMe	—	—	3.92	—	3.88
ArOH	?	6.08 bs		6.38 bs	—

[a]In pyridine-d_5.
[b]In $CDCl_3$.
[c]Intensity increased by a factor of 2.3 at 50°.
[d]Intensity increased by a factor of 1.4 at 50°.

The low resolution mass spectrum of **2** (Scheme 1) was noticeably different from that of sugiol (**1**) (10), but the high resolution exact mass measurement of all ions greater than 5% of the base peak indicated a close relationship between the two. Where indicated with m, the transistions shown in Scheme 1 were substantiated by metastable peaks. The presence of all four oxygen atoms in the fragment ions at *m/z* 236 and *m/z* 234 in **2**, corresponding to ions at *m/z* 218 and *m/z* 216 with both oxygen atoms in **1**, respectively, shows ring A in **2**, as in **1**, to be unoxidized. Similarly, the finding of the base peak of *m/z* 203 in **2** with the same elemental composition as for the same ion peak in **1**, and the presence of significant peaks at *m/z* 300 (M-H_2O) and *m/z* 274 (M-CO_2) strongly support structure **2**.



SCHEME 1. Major fragment ions (*m/z* ratios) in the mass spectrum of Cupresol (**2**)

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

C	**1**[a]	**2**[b]
1	38.0 t	38.1
2	19.1 t	18.6
3	41.5 t	36.7 (br)
4	33.3 s	41.7 (br)
5	49.9 d	116.6 s
6	36.3 t	—
7	197.2 s	165.7
8	134.1 s	134.0
9	156.6 s	147.8
10	38.0 s	43.4
11	110.1 d	110.5
12	161.7 s	161.1
13	124.0 s	124.0
14	126.6 d	129.0
15	27.3 d	27.0
16	22.6 q*	22.5
17	22.8 q*	22.5
18	32.6 q	26.2*
19	21.3 q (br)	25.8* (br)
20	23.3 q	25.8*

[a]In pyridine-d_5
[b]In pyridine-d_5-$CDCl_3$

Methylation of **2** with CH_2N_2 yielded ketoester **4**, no doubt via the ketoacid tautomer of **2**. The ir spectrum of **4** shows characteristic bands for hydroxyl, cyclohexanone, and conjugated carboxyl ester groups. Its ^{1}H-nmr parameters are given in Table 1. Its mass spectrum which exhibited an abundant $M^{+\cdot}$ at *m/z* 332 was quite different from that of **2**. The genesis of three prominent peaks in the upper mass region at *m/z* 304, *m/z* 261, and *m/z* 233 (base) can be rationalized as shown in Scheme 1.

Methylation of **2** with methyl iodide gave a mixture of **4** and its further methylated analog **5**; the structure of the latter was deduced from its ir (no hydroxyl band), ^{1}H-nmr (Table 1), and mass spectra [peaks at *m/z* 346 ($M^{+\cdot}$), 318, 275, and 247 all shifted by 14 mass units, corresponding to ions shown in Scheme 1].

EXPERIMENTAL

The plant material used in this work was collected in Washington during November 1968. Identification was confirmed by Dr. Robert E. Perdue, Medicinal Plant Resources Laboratory, U.S. Department of Agriculture, Beltsville, MD, where a reference specimen (PR 17446) is maintained.

GENERAL EXPERIMENTAL PROCEDURES.—For the instrumental procedures used, see S.D. Jolad, *et al. J. Org. Chem.*, **46**, 4267 (1981).

ISOLATION OF SUGIOL (**1**) AND CUPRESOL (**2**).—The powdered stem bark of *C. abramasiana* (6 kg) was extracted in a Lloyd extractor with ligroin for 48 h and then with 95% EtOH for 72 h. Evaporation of the solvent from the EtOH extract left a residue that was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ soluble portion, after evaporation of the solvent, was separated into Et_2O soluble and insoluble fractions. The Et_2O soluble residue upon treatment with CH_3CN gave a residue that we filtered out. The CH_3CN soluble residue was further separated into Et_2O soluble and insoluble fractions. The Et_2O soluble residue was then placed on the top of an EM SiO_2-60 short column and eluted successively with CH_2Cl_2, Et_2O, and MeOH. The fraction eluted with Et_2O was vacuum dried and redissolved in a small amount of Et_2O. On cooling, sugiol (**1**) separated as brilliant, golden-yellow rectangular prisms. The ether-soluble mother liquor, after removal of the solvent under vacuum, was subjected to EM SiO_2-60 column chromatography. The column was eluted with CH_2Cl_2 containing gradually increasing amounts of EtOAc. Fractions which showed essentially a single spot, corresponding to cupresol (**2**), were combined and purified twice by preparative tlc (SiO_2-60 PF-254) using CH_2Cl_2-EtOAc (94:6) as the developing solvent.

SUGIOL (**1**).—Crystallization from pyridine-MeOH and recrystallization from EtOH afforded sugiol (**1**) as colorless lustrous rectangular prisms, mp 286-288° [lit. 283-284° (1,2), 292-294° (6)], $[\alpha]^{25}D$ +27.9° (c 4.17, C_5H_5N) [lit. +34.4° (C_5H_5N) (1,2), +26° (EtOH) (5,7), +36° ($CHCl_3$+C_5H_5N) (8,9)]. The ir [(KBr) 3110 (very broad), 1635, 1600, 1585, 1560, 1495, 1450, 1370, 1335, 1305, 1265, 1170, 1080, 900, 860, 765 cm^{-1}], nmr (Tables 1 and 2), and mass spectra (10) were in accord with structure **1**. Sugiol (**1**) was inactive against *in vivo* 3PS and *in vitro* 9KB tumor systems.

SUGIOL ACETATE (**1a**). Compound **1a** was prepared from Ac_2O-pyridine (25°, 24 h) and crystallized from MeOH as lustrous long rectangular prisms, mp 164-165° [lit. 164-165° (7)], $[\alpha]^{25}D$ +29.4° (c 3.9, $CHCl_3$) [lit. +26.7° (1,2)]. The ir spectrum [(KBr) 1752, 1675, 1203 cm^{-1}] was in accord with structure **1a**.

CUPRESOL (**2**). Compound **2** was a colorless foam, $[\alpha]^{25}D$ +48.2° (c 0.76, $CHCl_3$), homogenous by tlc. The ir [($CHCl_3$) 3600, 3300, 3010, 1685, 1610, 1585, 1500, 1460, 1430, 1380, 1315, 1265, 1110, 1085, 1030, 1005, 960, 860 cm^{-1}], ^1H-nmr (Table 1), ^{13}C-nmr (Table 2) and mass [*m/z* 318 ($M^{+\cdot}$, 29), 303 (1.4), 301 (1.4), 300 (5.6), 285 (3.1), 274 (4), 256 (1.3), 247 (5.1), 236 (4), 234 (3), 232 (4.1), 231 (3.6), 229 (3.6), 219 (12.8), 218 (9), 217 (7.5), 216 (3), 213 (18.9), 206 (13.3), 203 (100), 190 (10.9), 189 (10.6), 187 (9.4), 175 (6.5), 164 (11.2), 163 (9), 161 (13.5), 159 (10.1), 147 (11.5), 145 (7.1), 133 (4.9), 119 (17), 117 (22.4), 115 (10.5), 107 (5.8), 91 (9.6), 69 (13), 57 (10.6), 55 (17.6)] spectra were in accord with structure **2**. Cupresol (**2**) was inactive against *in vivo* 3PS and *in vitro* 9KB tumor systems.

Anal. calcd for $C_{19}H_{26}O_4$: MW, 318.1831. Found: MW, 318.1833 (hrms).

METHYL (R)-2-(1,3,3-TRIMETHYL-2- OXOCYCLOHEXYL)-4-HYDROXYL-5-ISOPROPYLBENZOATE (**4**).—Compound **4** was obtained by treatment of **2** with CH_2N_2 followed by separation from the reaction mixture by preparative tlc [SiO_2-60 PF-254; CH_2Cl_2-EtOAc (80:20)] as colorless needles from isopropyl ether, mp 215-216°. The ir [(KBr), 3300, 1710, 1665, 1250 cm^{-1}], ^1H-nmr (Table 1) and mass [*m/z* 332 ($M^{+\cdot}$, 26.1), 304 (21.2), 273 (4.9), 272 (6.2), 261 (73.1), 233 (100), 229 (23.2), group of intense peaks between 222-216, 207-201, and 191-187, 175 (11.3), 173 (12.7), 163 (20), 161 (10.1), 159 (38.3), 147 (10.2), 145 (10.5), 128 (10), 115 (16.9), 91 (15.8), 77 (11.3), 69 (16.7), 59 (10), 55 (26.2)] spectra were in accord with structure **4**.

Anal. calcd for $C_{20}H_{28}O_4$: MW, 332.1988. Found: MW, 332.1988 (hrms).

METHYL (R)-2-(1,3,3-TRIMETHYL-2-OXOCYCLOHEXYL)-4-METHOXY-5-ISOPROPYLBENZOATE (**5**). —Methylation of **2** in dry Me_2CO with CH_3I in the presence of anhydrous K_2CO_3 at 56-60° for 4 h yielded a mixture of two components which were separated by preparative tlc [SiO_2-60 PF-254; CH_2Cl_2-EtOAc (94:6)]. The lower Rf component was identical in all respects with **4**. The higher Rf component, crystallized from hexane, had mp 112-113°. The ir [(KBr) 1712, 1688, 1250 cm^{-1}], ^1H-nmr (Table 1) and mass [*m/z* 346 ($M^{+\cdot}$, 4.9), 318 (4), 275 (8.4), 247 (16.6), group of peaks between 236-230, 221-215 and 201-204, 177 (4), 173 (5.1), 150 (7.2), 135 (15.3), 91 (8.4), 82 (100), 77 (7.3), 67 (7.8), 55 (10.6), 54 (31.6), 53 (10.1)] spectra were in accord with structure **5**.

Anal. calcd for $C_{21}H_{30}O_4$: MW, 346.2144. Found: MW, 346.2134 (hrms).

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