ISOIGUESTERIN, A NEW ANTILEUKEMIC BISNORTRITERPENE FROM *SALACIA MADAGASCARIENSIS*

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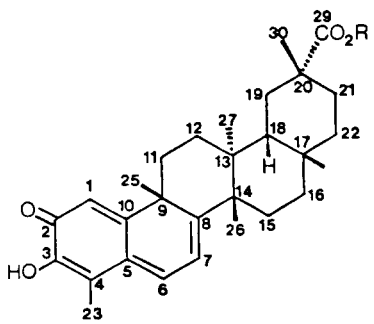
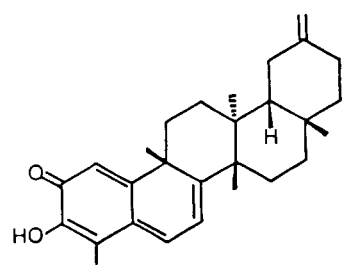
Pentacyclic nortriterpene quinone methides such as celastrol (**1**) and pristimerin (**2**) are commonly isolated from several genera of the Celastraceae family, including various *Salacia* species (1). Pristimerin (**2**) and maitenin, a related bisnortriterpene quinone methide, have both been reported to have antitumor activity (2, 3). An investigation of *Salacia madagascariensis* (Lam.) DC (Celastraceae) guided by activity against the P388 lymphocytic leukemia *in vivo* (4) has now yielded the new antileukemic pentacyclic bisnortriterpene quinone methide isoiquesterin (**3**).



1 R: H

2 R: CH_3

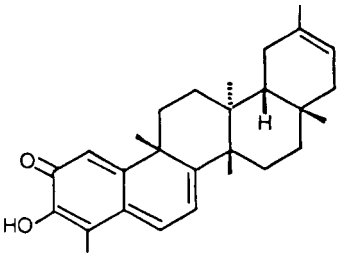


3

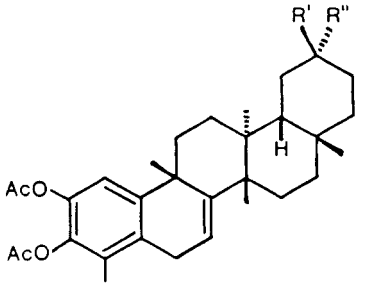
Initially, a room-temperature 95% ethanol extract of *S. madagascariensis* was subjected to an extensive fractionation procedure to yield **3**.[1] Subsequently, a more effective isolation procedure was developed. The dried, ground roots were extracted with petroleum ether (30°–75°) at room temperature in two separate 24 hr. extractions. The dark red oil resulting from the combined extracts was subjected to column chromatography over Silica Gel 60 (EM Labs) and eluted with dichloromethane followed by dichloromethane containing increasing amounts of ethyl acetate. Two consecutive fractions which eluted with 1–2% ethyl acetate in dichloromethane were combined and evaporated. The resulting orange solid was crystallized from ethanol-water to yield **3**.

High resolution mass spectrometry and elemental analysis established a molecular formula of $C_{28}H_{36}O_2$ for isoiguesterin (**3**). This molecular formula is identical to that of iguesterin (**4**), a pentacyclic bisnortriterpene quinone methide isolated from *Catha cassinoides* (5). The mass spectral fragmentation pattern of **3** showed major ions at *m/e* 389, 253, 241, 202, 201, 200, 187, 147, 95, as did that of celastrol (**1**). These also correspond to the major ions reported for **4** (5). The ultraviolet spectrum [λ max (ε)

[1]The initial isolation was carried out in the laboratories of the late Dr. S. Morris Kupchan at the University of Virginia by Dr. Y. Shizuri.



4



5 R',R": $=CH_2$

6 R': CH_3,
R": CO_2H

254 nm (sh) (6320), 422 (9420)] of **3** was similar to the spectra of celastrol (**1**) and iguesterin (**4**) (5) indicating that all three compounds contain similar chromophores. The infrared spectrum of **3** was also similar to the spectra of **1** and **4** (5) with a carbonyl band at 1595 cm^{-1} and an -OH band at 3400 cm^{-1}, The major difference between the spectra of **3** and **1** was the absence of any carbonyl peak in the spectrum of **3** which would correspond to a C-20 acid moiety (1705 cm^{-1} in the spectrum of **1**).

The nature of the chromophore was confirmed by the pmr spectrum of **3**. The C-7, C-1, and C-6 protons appeared as a one proton doublet at δ6.32, a one proton doublet at δ6.53, and a one proton doublet of doublets at δ7.01, respectively. This pattern is identical to that found in the spectra of celastrol **1** [δ6.32 (d), 6.50 (d), 7.05 (dd)] and iguesterin **4** [δ6.34 (d), 6.65 (s), 7.03 (d)][2] (5).

Also evident in the pmr spectrum of **3** were five singlets at δ0.70, 1.16, 1.29, 1.47, and 1.21 which were assigned to the C-13, C-17, C-14, C-9, and C-4 methyl groups, respectively, by comparison to the reported data for **4** (5). However, the doublet at δ1.61 assigned to the C-20 methyl group and the multiplet at δ5.24 assigned to the C-21 proton in the spectrum of **4** were not present in the spectrum of **3**. Instead of these signals, a broad, two proton singlet at δ4.58 suggested the presence of an exocyclic double bond in **3**. Based on these data and on biogenetic grounds structure **3** is proposed for isoiguesterin.

Additional support for the structure of **3** was obtained from the cmr spectrum. By comparison of the completely decoupled, partially relaxed and off-resonance decoupled spectra of **3** to similar data obtained for celastrol **1** and published data for pristimerin (**2**) (6), many of the signals in the spectrum of **3** could be assigned (table 1). The spectra of **1** and **2** (6) each showed a signal for the C-20 methyl group (at 30.7 and 30.8 ppm, respectively) and signals for five quaternary carbons between 30 and 45 ppm, as well as a signal for the C-20 carboxyl moiety. In the spectrum of **3**, however, only four quaternary carbons appear between 31.7 and 44.9 ppm. The fifth quaternary carbon appears at 148.0 ppm, indicating that it is now involved in a double bond. The signal for the C-20 methyl group is also absent, having been replaced by a signal at 108.3 ppm which appears as a triplet in the off-resonance

[2]The relatively small 1,6 coupling constant (J=1.5 Hz) was most likely not observed in the 60 MHz spectrum.

TABLE 1. Cmr peak assignments (shifts in ppm).

	C	*2* (Ref. 6)	*1*	*3*
$-CH_3$.....	23	10.2	10.5	10.4
	25	38.2	38.4	39.0
	26	21.5	21.5	21.3
	27	18.3	18.7	19.7
	28	31.5	31.5	31.2
	30	30.8	30.7	—
	31	51.4	—	—
$>CH_2$.....	11	28.6	28.7	28.5
	12	29.6	29.3	29.8
	15	29.8	29.5	30.5
	16	30.4	32.4	30.6
	19	33.5	33.8	34.0
	21	34.8	34.5	36.1
	22	36.3	36.3	37.0
	30	—	—	108.3(t)
$\gtrless C-H$.....	1	119.5	120.6	119.6
	6	133.8	135.4	134.0
	7	118.0	118.3	117.9
	18	44.2	44.2	45.0
$-\overset{\vert}{\underset{\vert}{C}}-$.....	3	117.1	120.4	117.2
	4	127.2	127.5	127.5
	5	146.0	147.0	146.1
	8	164.0	165.0	165.2
	10	169.8	172.6	170.3
	20	30.5	31.0	148.0(s)
	9	38.4	39.3	31.7
	13	39.3	39.9	41.4
	14	40.3	43.1	43.0
	17	44.9	45.3	44.9
$>C=O$....	2	178.4	178.3	178.4
	29	178.1	182.5	—

decoupled spectrum. The signal for the C-20 acid carbonyl is absent as well. These data thus confirm the presence of an exocyclic double bond at C-20 in **3**.

Further evidence for structure **3** was obtained from reduction of the chromophore. Isoiguesterin (**3**) was treated with sodium borohydride in ethanol at room temperature followed by acetic anhydride-pyridine to give dihydroisoiguesterin diacetate **5** (5, 7). The pmr spectrum of **5** exhibited the expected signals for the C-1, 6, and 7 protons, five methyl groups, two acetate moieties, and the exocyclic double bond (δ4.54, br s). The cmr spectrum also contained the expected signals for the exocyclic double bond at 107.9 ppm and 148.4 ppm which were not present in the spectrum of dihydrocelastrol diacetate (**6**).[3]

Isoiguesterin (**3**) exhibited activity *in vivo* against the P388 lymphocytic leukemia in mice at two dose levels (T/C 147 at 1.00 mg/kg, 125 at 0.75 mg/kg) in initial testing and *in vitro* against the KB cell culture (ED_{50} 0.24 μg/ml) (4). Subsequent testing has shown that the toxicity of **3** which occurs at doses above 1.0–2.0 mg/kg often overrides any therapeutic effect.

[3]Dihydrocelastrol diacetate was prepared as in Reference 7.

EXPERIMENTAL[4]

PLANT MATERIALS.—Dried roots of *Salacia madagascariensis* (Lam.) DC (B811595, PR-80767), collected in Tanzania in March, 1974, were supplied by the Medicinal Plant Resources Laboratory, USDA, Beltsville, Maryland, where voucher specimens are preserved.

EXTRACTION AND PRELIMINARY FRACTIONATION.—The dried, ground roots of *S. madagascariensis* (1.9 kg) were extracted with 8 liters of petroleum ether (30°–75°) at room temperature for 24 hr., followed by a second 24 hr. extraction with fresh solvent. The combined extracts were evaporated under reduced pressure to give fraction A. (16 g). Fraction A was subjected to column chromatography over silica gel 60 (800 g) with dichloromethane followed by dichloromethane containing increasing amounts of ethyl acetate as eluent. Fractions B (600 mg) and C (400 mg) eluted with 1% ethyl acetate in dichloromethane.

ISOLATION OF ISOIGUESTERIN (**3**).—The material from fraction B was crystallized from benzene-hexanes and the material from fraction C was crystallized from 95% ethanol. The crystalline materials were combined and recrystallized from 95% ethanol to yield orange crystals of isoiguesterin (**3**), 345 mg. The mother liquors from all crystallizations were combined and subjected to a short column chromatography over silica gel 60 eluted with dichloromethane to obtain an additional 180 mg **3**. Total yield of **3**, 525 mg, 0.028%; mp 203–205°; uv max (EtOH) λ 254 nm (sh, ε 6320), 422 (9420); ir (KBr) 3400, 2940, 1595, 1555, 1520, 1485, 1285, 1215 cm^{-1}; pmr ($CDCl_3$) δ 0.70 (3H, s, 13-CH_3), 1.16 (3H, s, 17-CH_3), 1.29 (3H, s, 14-CH_3), 1.47 (3H, s, 9-CH_3), 2.21 (3H, s, 4-CH_3), 4.58 (2H, br s, 20- C=CH_2), 6.32 (1H, d, J=7 Hz, 7-H), 6.53 (1H, d, J=1.5 Hz, 1-H), 7.01 (1H, dd, J=1.5 and 7 Hz, 6-H); mass spectrum *m/e* 404, 389, 253, 241, 202, 201, 200, 187, 147, 95; high resolution mass spectrum *m/e* 404.2707 (M^+, calcd for $C_{28}H_{36}O_2$, 404.2715).

Anal. Calcd for $C_{28}H_{36}O_2$: C, 83.12; H, 8.97. Found: C, 83.11; H, 9.00.

DIHYDROISOIGUESTERIN DIACETATE (**5**).—A solution of 54.3 mg of isoiguesterin (**3**) in 3 ml of ethanol was treated with 17 mg sodium borohydride. The bright red-orange solution immediately changed to pale yellow with vigorous bubbling. The solution was stirred at room temperature for 1 hr. The excess borohydride was destroyed with 4 drops of acetic acid, and the pale yellow solution was evaporated to an oily solid. This solid was suspended in 15 drops of dry pyridine and 15 drops of acetic anhydride, and the resulting mixture was stirred at room temperature for 24 hr. The pyridine was evaporated at reduced pressure, the residue suspended in dichloromethane and filtered. The filtrate was evaporated to a brown oil which was subjected to ptlc on silica gel 60 developed in dichloromethane. This afforded a clear glass which was crystallized from ethanol to pale-yellow crystals of **5**, 30.2 mg; mp 165–168°; uv (EtOH) λ (ε) 268 nm (370); ir ($CHCl_3$) 2940, 1770, 1605, 1460, 1370, 1160, 900 cm^{-1}; pmr ($CDCl_3$) δ 0.73 (3H, s, 13-CH_3), 1.14 (3H, s, 17-CH_3), 1.24 (3H, s, 14-CH_3), 1.36 (3H, s, 9-CH_3), 2.06 (3H, s, 4-CH_3) 2.26 (3H, s, -OAc), 2.30 (3H, s, -OAc), 3.13 (1H, d, J=3.2 Hz, 6-H), 3.25 (1H, d, J=5.4 Hz, 6-H), 4.54 (2H, br s, C=CH_2), 5.69 (1H, dd, J=3.2 and 5.4 Hz, 7-H), 7.00 (1H, s, 1-H); mass spectrum *m/e* 490(M^+), 475, 271, 229, 187, 95.

[4]All mps were obtained on a Fisher-Johns melting point apparatus and are uncorrected. The pmr and cmr spectra were recorded on a JEOL FX90QII spectrometer with TMS as an internal standard. The ir spectra were measured on a Perkin-Elmer model 283 instrument and the uv spectra were measured on a Beckman Acta MVII recording spectrophotometer. The low resolution mass spectra were measured on a Finnigan model 4000 spectrometer. The high resolution mass spectra were obtained at the University of Pennsylvania Mass Spectrometry Center. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Michigan, and Atlantic Microlab, Inc., Atlanta, Georgia. The P388 *in vivo* assays were performed at Raltech Associates, Madison, Wisconsin, and the 9KB cytotoxicity assays were performed at Arthur D. Little, Inc., Cambridge, Massachusetts.

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LITERATURE CITED

1. R. Brüning and H. Wagner, *Phytochemistry*, **17**, 1821 (1978).
2. E. Schwenk, *Arzneimittelforschung*, **12**, 1143 (1962).
3. F. Delle Monache, G. B. Marini-Bettolo, O. Goncalves de Lima, I. Leonicio D'Albuquerque, and J. Sydney de Barros Coelho, *Gazz. Chim. Ital.*, **102**, 317 (1972).
4. R. I. Geran, N. H. Greenberg, M. M. McDonald, A. M. Schumacher and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972).
5. A. G. Gonzalez, C. G. Francisco, R. Freire, R. Hernandez, J. A. Salazar, and E. Suarez, *Phytochemistry*, **14**, 1067 (1975).

6. K. Nakanishi, V. P. Gullo, I. Miura, T. R. Govindachari, and N. Viswanathan, *J. Amer. Chem. Soc.*, **95**, 6473 (1975).

7. K. Nakanishi, Y. Takahashi, and H. Budzikiewicz, *J. Org. Chem.*, **30**, 1729 (1965).