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A DITERPENE FROM *GARBERIA HETEROPHYLLA*

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Key Word Index—*Garberia heterophylla*; Asteraceae; Eupatorieae; diterpene; garberic acid; sesquiterpene lactone, guaianolide, eupahakonesin.

Abstract—The aerial parts of *Garberia heterophylla* afforded the known guaianolide-type sesquiterpene lactone eupahakonesin, 2,6-dimethoxybenoquinone and the triterpene epifriedelinol. In addition, a new and novel diterpene, garberic acid, was isolated. Its structure and stereochemistry was established by spectral methods and single crystal X-ray diffraction studies.

INTRODUCTION

As part of our chemical study of members of the Florida Scrub community [1], we have investigated *Garberia heterophylla* of the tribe Eupatorieae, an evergreen shrub endemic to the Florida scrub [2]. The petrol extract of dried aerial parts of *G. heterophylla* afforded the known guaianolide-type sesquiterpene lactone, eupahakonesin (2) [3], 2,6-dimethoxybenzoquinone [4] and the triterpene epifriedelinol [5]. Compound **2**, which had been previously obtained from *Eupatorium chinense*, exhibited ^{1}H and ^{13}C NMR spectral data in full agreement with those reported for eupahakonesin [3]. The structure and relative stereochemistry of the new diterpene (**1a**), which we named garberic acid, was determined by single crystal X-ray diffraction studies, and its ^{1}H and ^{13}C NMR spectra were assigned by combined applications of COSY, ^{1}H,^{13}C-correlation, DEPT and INEPT methods [6, 7].

RESULTS AND DISCUSSION

The diterpene **1a**, the major constituent of the leaves of *G. heterophylla*, gave a molecular ion at *m/z* 336. The ^{13}C NMR spectrum of **1a** indicated the presence of 20 carbons with four methyls, seven methylenes, four methine and five quaternary carbons (Table 1), the assignments being made by COSY, ^{1}H,^{13}C-correlation and DEPT methods. The ^{1}H NMR spectrum integrated for 32 hydrogens, which was in accord with a molecular formula $C_{20}H_{32}O_4$. Broad IR absorptions at 3500–2400 cm^{-1} as well as NMR spectroscopic data suggested the presence of carboxylate group(s). Methylation of **1a** with excess diazomethane in ether yielded the diester **1b**, which was analysed by GC-mass spectrometry, IR and NMR spectral methods. The ^{1}H NMR spectrum of **1b** gave three methyl signals at δ0.79, 1.64 and 1.73, which were assigned to a methyl group at a quaternary carbon and two olefinic methyls, respectively. Furthermore,

Table 1. ^{13}C NMR spectral data of diterpenes **1a** and **1b** (100 MHz, TMS as int. standard)

C	1a*	1b*
1	47.1 *d*	47.2 *d*
2	135.7 *s*	135.7 *s*
3	121.7 *d*	121.7 *d*
4	28.9 *t*	28.7 *t*
5	48.8 *d*	48.8 *d*
6	38.7 *s*	38.6 *s*
7	29.4 *t*	29.3 *t*
8	38.6 *t*	38.6 *t*
9	31.1 *d*	31.2 *d*
10	41.2 *t*	41.4 *t*
11	179.6 *s*[a]	173.4 *s*[a]
12	25.4 *t*	25.0 *t*
13	32.2 *t*	32.6 *t*
14	180.7 *s*[a]	174.4 *s*[a]
15	16.0 *q*	16.1 *q*
16	147.4 *s*	147.4 *s*
17	113.8 *t*	113.7 *t*
18	22.8 *q*	22.8 *q*
19	22.4 *q*	22.4 *q*
20	19.7 *q*	19.5 *q*
1′		51.2 *q*[b]
2′		51.4 *q*[b]

Peak multiplicities were obtained by heteronuclear multipulse programs (DEPT).

[a,b]Interchangeable within a column.

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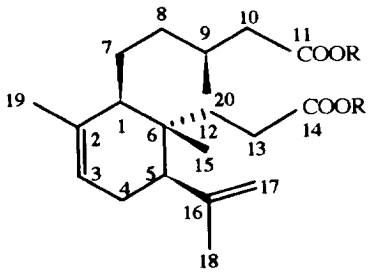
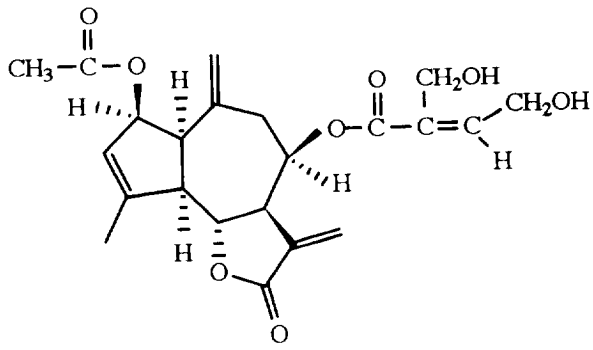


1a R=H

1b R=CH_3



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a methyl doublet at δ0.91 (J = 6.7 Hz) and two overlapping methoxy singlets at δ3.61 appeared as distinct signals. The presence of an olefinic methylene group was suggested by two broad singlets at δ4.72 and 4.78.

Because the diterpene **1a** and its diester **1b** exhibited strongly overlapping ^{1}H NMR signals in major parts of the spectrum, detailed structural assignments by modern 2D NMR methods were difficult. Therefore, due to the availability of suitable crystals of garberic acid (**1a**), a single crystal X-ray diffraction analysis of **1a** was performed. This allowed for determination of the molecular structure and also gave the relative stereochemistry of the molecule, which is shown in Fig. 1. The precision of the crystal structure determination of **1a** is limited by the high thermal parameters in general, and, in particular, by disorder of both carboxyl groups and the group C-16 through C-18, in which the single and double bonds are superimposed by two-fold rotation about the C-5/C-16 bond. However, the relative configurations of all the stereogenic centres are established, as is the conformation of the molecule, as shown in Fig. 1. Both COOH groups are involved in intermolecular hydrogen bond dimers.

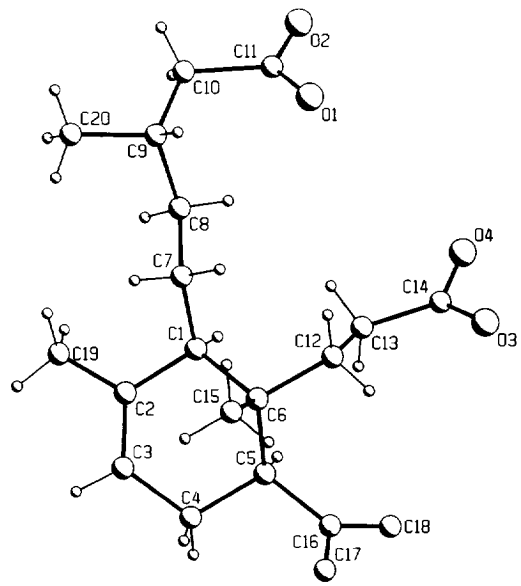
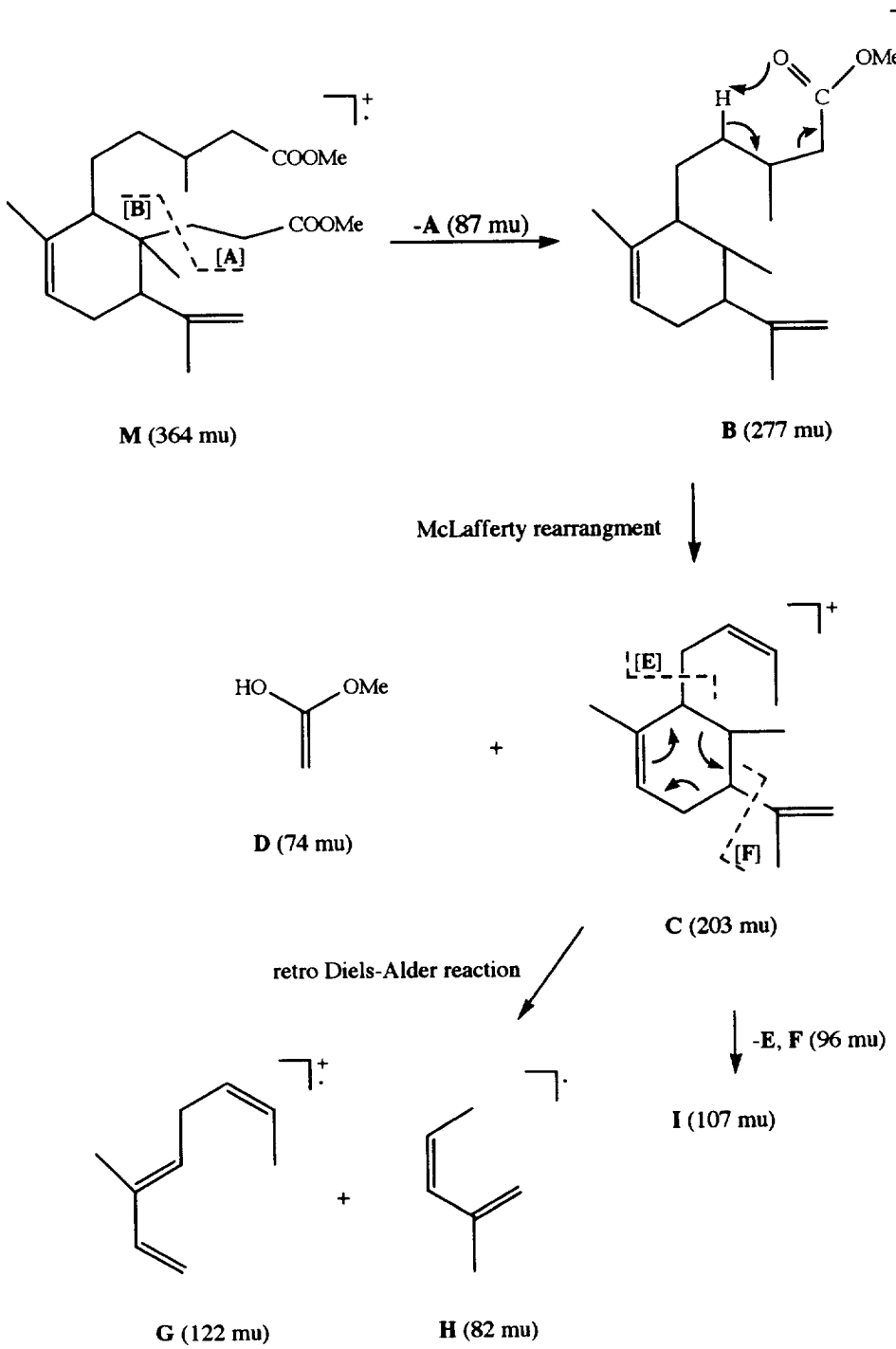


Fig. 1. The molecular structure of garberic acid (**1a**).

Although detailed experimental evidence is lacking, a reasonable fragmentation pattern of **1b** is proposed in Scheme 1. The EI-mass spectrum of **1b** showed a molecular ion at m/z 364. A strong peak at m/z 277 $[\mathbf{B}]^+$ suggested the loss of fragment **A** ($CH_2CH_2CO_2Me$), from the parent ion. A strong peak at m/z 203 $[\mathbf{C}]^+$ must be derived from the McLafferty rearrangement of fragment $[\mathbf{B}]^+$ by loss of **D** (74 mu). Further fragmentation of cation **C** by a retro-Diels–Alder process would give radical ions **G** (122 mu) and **H** (82 mu), a fact which was supported by a strong peak at m/z 122. Alternatively, loss of fragments **E** (55 mu) and **F** (41 mu) from $\mathbf{C}^+$ would lead to fragment **I**, as indicated by the intense peak at m/z 107.

The ^{13}C NMR spectral assignments of **1a** and **1b**, which involved ^{1}H,^{13}C-correlations and DEPT experiments, are summarized in Table 1 and their ^{1}H NMR spectral data are given in the Experimental section. The INEPT method [6] was applied for the assignment of the quaternary olefinic carbons C-2 and C-16 in **1b**. Irradiation of the two protons at C-17 enhanced the signal at δ147.4 (C-16). Therefore, the other quaternary carbon signal at δ135.7 was assigned to C-2.

The skeletal arrangement of garberic acid is novel and, to our best knowledge, finds no structural analogues in the family Asteraceae or in other higher plant families. The closest structural relatives of the garberic acid skeleton seem to be the eunicellanes, which occur in a variety of soft corals (*Cladiella* and *Sclerophytum* species) and gorgonians (*Astrogorgia*, *Eunicella* and *Muricella* species) [8]. The eunicellanes, which represent non-rearranged cyclization products of geranylgeranyl pyrophosphate, are typically substituted and oxidatively modified bicyclo [4.8.0]tetradecanes (**J**). Biogenetically, the garberic acid skeleton could be formulated as a seco-eunicellane (**K**) formed by oxidative cleavage of the allylic carbons in the 10-membered ring of **J**. Furthermore, in the biosynthetic



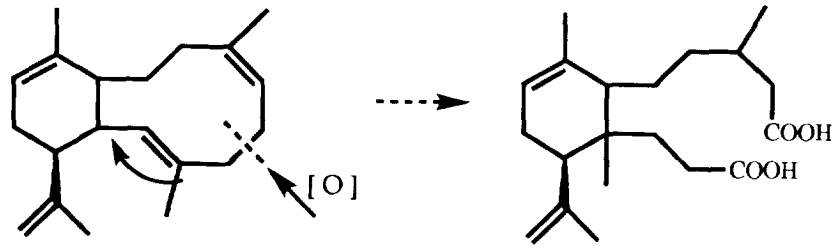
Scheme 1. Mass spectral fragmentation of **1b**.

transformation the methyl group at C-13 must undergo a shift to the C-6 position, as outlined in Scheme 2.

EXPERIMENTAL

General. ^{1}H NMR, COSY, inverse ^{13}C–^{1}H-correlation, long-range ^{13}C–^{1}H correlation, ^{13}C NMR and DEPT were obtained on a Bruker AM-400 spectrometer with $CDCl_3$ as solvent and TMS as int. standard. IR spectra were recorded on a Perkin Elmer 1760X FT-IR spectrometer using films on KBr plates. The optical rotation was recorded on a Jasco DIP-370 digital polarimeter.

Plant material. Aerial parts of *Garberia heterophylla* (Bartr.) Merr.& Harp were collected on 12 August 1992 North of Sun-N-Lakes, Highland County, Florida.



J K

Scheme 2. Proposed biogenic relationship between the garberic acid skeleton (**K**) and the eunicellanes (**J**).

A voucher is deposited at the Louisiana State University Herbarium (N. H. Fischer and H. D. Fischer No 453).

Extraction and isolation. Fresh leaves (101 g) were separated from the stems and soaked with 2 l of H_2O. The combined H_2O extracts were re-extracted with CH_2Cl_2 to give 0.15 g of extract. Then the plant material was soaked in petrol overnight and the extract evapd *in vacuo* to yield 2.5 g crude extract. The residue was subsequently soaked in hexane (2 l), CH_2Cl_2(2 l) and MeOH (2 l) yielding 2 g, 3 g and 2.5 g crude extracts, respectively. The petrol extract (50 mg) was dissolved in about 1 ml of $CDCl_3$; from this solution crystalline **1a** (3 mg) formed slowly. The stems (50 g) were soaked with 2 l of CH_2Cl_2 for 24 hr and the extracts evapd *in vacuo* to yield 1.1 g of crude extract.

Part of the petrol extract (1 g) from leaves was chromatographed over silica gel using VLC [9]. Elution with hexane–EtOAc mixts of gradually increasing polarity yielded 80 frs of 20 ml each. Frs 68–73, which were eluted with hexane–EtOAc (2:3) were combined and rechromatographed using VLC on silica gel. Subfrs 24′–26′, when eluted with hexane–EtOAc (3:7), yielded 3 mg of **2**.

The CH_2Cl_2 extract from stems was chromatographed over silica gel using VLC. Fr. 16, which was eluted with hexane–EtOAc (17:3) yielded epifriedelinol (20 mg), the structure of which was confirmed by NMR and measurement of the unit-cell dimensions, using single crystal X-ray diffraction [5]. Frs 52–60 contained 2,6-dimethoxybenzoquinone [4], the structure of which was confirmed by comparison of NMR and GC-MS data with reported values [5].

Esterification with diazomethane. Freshly distilled diazomethane [10] in ether was combined with crude petrol extract of *G. heterophylla* (1 g). The solution was stirred for 1 hr, left overnight, and then evapd *in vacuo* on a rotary evaporator. The crude reaction product was chromatographed by VLC on silica gel. Frs that were eluted with hexane–EtOAc (9:1) were analysed by GC-MS with a mass spectral peak at *m/z* 364, which verified the molecular weight of the methyl ester **1b**.

Garberic acid (**1a**). $C_{20}H_{32}O_4$, crystals; mp 104–107°; $[\alpha]_D^{23}$ + 56° (MeOH; *c* 0.10); IR ν_{max}^{KBr} cm^{-1}: 3500–2400 (COOH), 1707 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ0.82 (3H, *s*, H-15), 0.98 (3H, *d*, *J* = 6.6 Hz, H-20), 1.68 (3H, *s*, H-19), 1.76 (3H, *s*, H-18), 4.77 (3H, *br s*, H-17a), 4.82 (3H, *br s*, H-17b); ^{13}C NMR see Table 1.

Table 2. Positional parameters of garberic acid (**1a**) and their estimated s.d.s

Atom	*x*	*y*	*z*	Beq (nm^2)
O-1	0.036(1)	0.5169(8)	0.0362(2)	0.152(2)
O-2	0.046(2)	0.3823(9)	−0.0202(2)	0.193(3)
O-3	0.554(2)	0.8824(8)	0.0644(1)	0.146(3)
O-4	0.569(2)	0.7264(8)	0.0114(1)	0.159(3)
C-1	0.501(1)	0.3643(9)	0.1436(2)	0.092(2)
C-2	0.573(1)	0.2399(9)	0.1726(2)	0.109(2)
C-3	0.700(2)	0.264(1)	0.2013(2)	0.135(3)
C-4	0.795(2)	0.416(1)	0.2069(2)	0.127(3)
C-5	0.752(2)	0.529(1)	0.1712(2)	0.116(2)
C-6	0.550(1)	0.5293(9)	0.1605(2)	0.091(2)
C-7	0.306(1)	0.3493(9)	0.1338(2)	0.095(2)
C-8	0.262(1)	0.275(1)	0.0923(2)	0.099(2)
C-9	0.064(1)	0.243(1)	0.0847(3)	0.133(3)
C-10	0.022(2)	0.234(2)	0.0411(4)	0.159(3)
C-11	0.037(2)	0.396(1)	0.0195(3)	0.144(3)
C-12	0.508(1)	0.6489(9)	0.1257(2)	0.089(2)
C-13	0.587(1)	0.618(1)	0.0812(2)	0.091(2)
C-14	0.573(1)	0.750(1)	0.0521(2)	0.095(2)
C-15	0.434(2)	0.566(1)	0.2002(2)	0.119(3)
C-16	0.842(2)	0.683(1)	0.1802(2)	0.125(3)
C-17	0.808(2)	0.756(2)	0.2221(3)	0.171(4)
C-18	0.951(2)	0.739(2)	0.1469(3)	0.151(4)
C-19	0.506(3)	0.089(1)	0.1685(2)	0.166(4)
C-20	0.007(3)	0.103(2)	0.1103(4)	0.196(5)

Dimethyl garberate (**1b**). $C_{22}H_{36}O_4$, gum; IR ν_{max}^{KBr} cm^{-1}; 1735 (–COOMe); EIMS (70 eV) *m/z* (rel. int.): 364 $[M]^+$ (18), 349 $[M-Me]^+$ (1.1), 333 $[M-MeO]^+$ (6.3), 277 $[M-A]^+$ (51), 203 $[C]^+$(35), 122 $[G]^+$ (69), 109 (100), 107 $[C-D-F]^+$ (81), 55 $[E]^+$ (76), 41 $[F]^+$ (63); ^{1}H NMR (400 MHz, $CDCl_3$): δ0.79 (3H, *s*, H-15), 0.91 (3H, *d*, *J* = 6.6 Hz, H-20), 1.64 (3H, *s*, H-19), 1.73 (3H, *s*, H-18), 4.72 (3H, *br s*, H-17a), 4.78 (3H, *br s*, H-17b); ^{13}C NMR see Table.1.

X-Ray data of garberic acid (**1a**). A crystal of dimensions 0.27 × 0.48 × 0.55 mm was used for data collection on an Enraf-Nonius CAD4 diffractometer equipped with CuKα radiation (= 0.154184 nm), and a graphite

monochromator. Crystal data are: $C_{20}H_{32}O_4$, M_r = 336.5, orthorhombic space group $P2_12_12_1$, a = 0.7413 (1), b = 8.858 (2), c = 3.1016 (6) nm, V = 2.037 (1) nm^3, Z = 4, dc = 1.097 g cm^{-3}, T = 2.4 . Intensity data were measured by w-2q scans of variable rate, designed to yield I = 50σ (I) for all significant reflections. An octant of data was collected within the limits 2 < q < 75°. Data reduction included corrections for background, Lorentz, polarization, decay and absorption effects. Absorption corrections (μ = 5.6 cm^{-1}) were based on ψ scans, with minimum relative transmission coefficient 86.9%. Intensity decay amounted to 11.9%. Of 2323 unique data, 1474 had I > 3σ(I) and were used in the refinement.

The structure was solved by direct methods and refined by full-matrix least squares, treating non-hydrogen atoms anisotropically, using the Enraf-Nonius MolEN programs [11]. Hydrogen atoms were placed in calculated positions, except for those on the COOH groups and C-17 and C-18, which could not be located because of the disorder. Convergence was achieved with R = 0.12. The crystal structure is illustrated in Fig. 1, and its coordinates are tabulated in Table 2. The limited precision of the determination precluded determination of the absolute configuration.

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