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A NEW CEDRENE ISOPRENOLOGUE FROM THE RESIN OF *EREMOPHILA GEORGEI*

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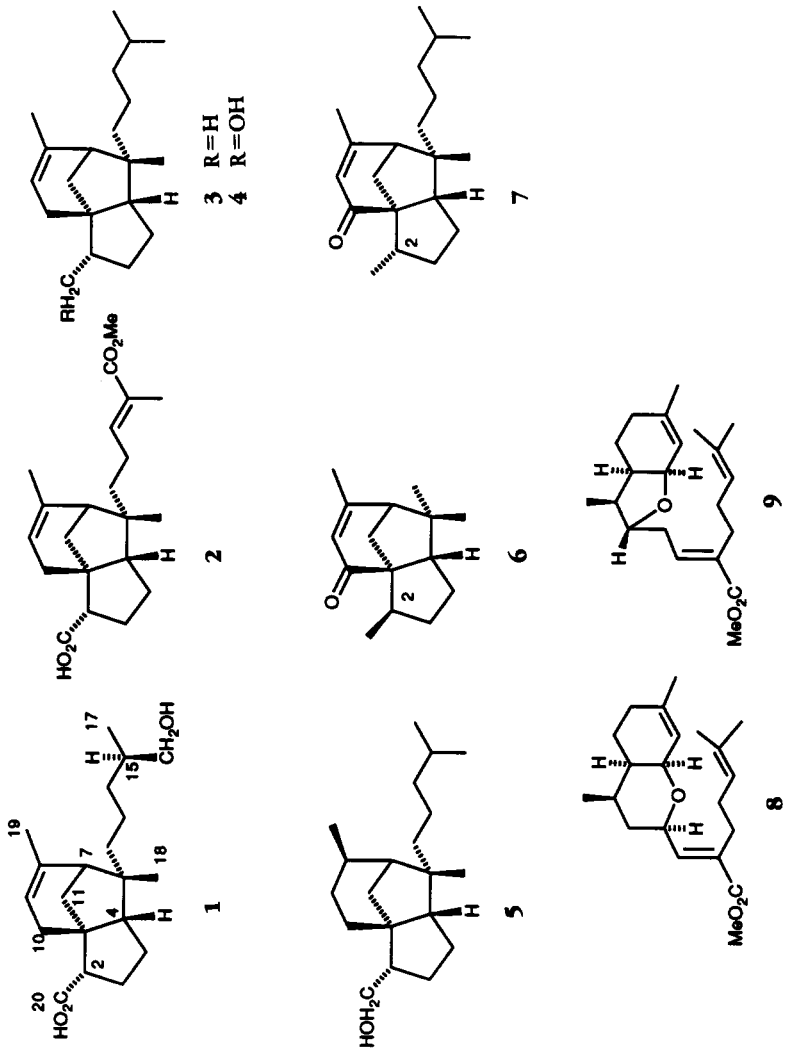
ABSTRACT.—The isolation of a new diterpene acid **2** from the resin of a variety of *Eremophila georgei* is described. The structure of the acid was identified by chemical and spectroscopic methods and was shown to contain a C_5-extended cedrene nucleus. The occurrence of cedrene and bisabolene isoprenologues from an unidentified *Eremophila* sp. is reported.

A number of *Eremophila* species (Myoporaceae) produce copious quantities of resin, which accumulates on their leaves and branchlets. Extensive studies have disclosed that these resins, in most cases, contain oxygenated diterpenes (1–6). Of the nine separate classes of diterpenes isolated from this source so far, four can be regarded as isoprenologues of the bisabolene (1), acorene (2), calamanene (3), and cedrene sesquiterpenes (4). Two others, namely the decipianes (5) and the eremanes (6), exhibit tricyclic nuclei which lack a naturally occurring sesquiterpene counterpart. The first and only example of a 2-*epi*-cedrene isoprenologue was isolated from a variety of *Eremophila georgei* Diels and was shown to have the structure and stereochemistry depicted in **1** (4). We now report on the structure of a second example **2** of this class and the co-occurrence of cedrene and bisabolene isoprenologues in another *Eremophila* species. We also describe the preparation of some useful derivatives from **2** and comment on nmr parameters which allow a distinction between the cedrene and the 2-*epi*-cedrene systems.

Extraction of a sample of *E. georgei* with Me_2CO and isolation of the acidic components by vlc (vacuum liquid chromatography) yielded two major fractions (4). The more polar fraction has been shown to contain the 2-*epi*-α-cedrene isoprenologue **1** and the corresponding exocyclic double bond isomer, but only the former could be obtained pure (4). The less polar, crystalline fraction appeared homogeneous by tlc, although the ^{1}H-nmr spectrum showed it to consist of one major (70%) and three minor components. Repeated chromatography gave fractions enriched in **2** which could be obtained in small amounts after repeated fractional crystallization from pentane.

The structure of this compound, $C_{21}H_{30}O_4$, was deduced from interpretation of the spectral data. The ^{1}H-nmr spectrum showed signals for a tertiary methyl group (δ_H 1.04, s), a vinylic methyl group (δ_H 1.67, d, $J = 1.5$ Hz) with coupling to a vinyl proton (δ_H 5.27, m), and a methoxyl group (δ_H 3.75, s). A signal at δ_H 6.78 (dt, $J = 1.3$, 6.1 Hz), coupled to a second vinylic methyl (δ_H 1.86, d, $J = 1.3$ Hz), was indicative of an α-methyl-α,β-unsaturated acid or ester group. The near coincidence of the chemical shifts for the carbons assigned to the tricyclic ring system in the ^{13}C-nmr spectrum of **1** (4) with those of **2** suggested a common nucleus. Evidence for this was obtained by converting the acid ester **2** by standard methods to the hydrocarbon **3**, which proved identical to a sample previously prepared from **1** (4), thus establishing the carbon skeleton and absolute stereochemistry of **2**.

The location of the methyl ester at C-16 was tentatively inferred from the similarity of the chemical shifts for C-1–C-5 in the spectra of **1** and **2**. [In the original report (4) on the structure of **1** an error in the interpretation of the ORTEP diagram led to C-15 being rep-


1
2
3 R=H
4 R=OH
5
6
7
8
9

resented with *S* configuration in the structural diagrams. In fact, **1** has the *R* configuration at this center. Also, for the ^{13}C-nmr spectrum of **1**, C-2 was quoted as δ 53.9 instead of δ 51.5.] Furthermore, the mass spectrum of **2** showed a base peak at *m/z* 114, indicating cleavage of the C-12–C-13 allylic bond with transfer of a hydrogen, consistent with the presence of a methyl ester on the side chain.

With this circumstantial evidence in mind, a more rigorous test was carried out by recourse to long-range ^{1}H-^{13}C decoupling measurements. By selective irradiation, the methoxyl protons were shown to be coupled to the signal at δ_C 168.7, which must be the carboxylate carbon. H-14 was also shown to be coupled to this carbon and to C-17 (δ_C 12.4). The conjugated double bond was assigned the *E* geometry since the vinylic proton resonated at δ_H 6.78, indicating deshielding by a cis carboxylic ester group, and the chemical shift of the carbon of the vinylic methyl (δ_C 12.4) showed it to be shielded by a cis carbon.

The acid ester **2** could be separated only with difficulty from three related compounds which, from nmr spectroscopy, were considered to be the exocyclic double bond isomer and the corresponding side-chain dihydro analogues; compound **2** was available in only small amounts. With a view to generating workable quantities of pure compounds containing this skeleton, a fraction enriched (70%) in the acid ester **2** was subjected to a reduction/hydrogenolysis sequence (see Experimental). From these transformations, the new derivatives **4** and **5** were characterized, and these, together with the hydrocarbon **3**, become useful markers to probe for the presence of neutral metabolites present in this and related plants.

During this work our attention was drawn to the fact that ^{13}C-nmr spectroscopy provides an unsatisfactory probe for the stereochemistry at C-2 in the cedrene nucleus. Comparison of the ^{13}C-nmr spectrum of α-cedrene with that of the diterpene hydrocarbon **3** (2-*epi*-cedrene nucleus) reveals only minor differences (up to 1.6 ppm; see Table 1) for C-1 to C-5, and even the methyl group at C-2 shows only slight shielding effects (1.7 ppm) in the 2-*epi*-cedrene nucleus. In connection with another project, we synthesized the conjugated enones in both series. In cedrenone **6**, the C-2 methyl protons resonate at δ_H 1.31, deshielded by 0.48 ppm compared to those in cedrene (δ_H 0.83). In the diterpene enone **7**, the corresponding methyl protons (δ_H 0.84) showed deshielding of 0.11 ppm compared to the hydrocarbon **3**. Molecular models clearly show that in the cedrene system the methyl group lies in the deshielding zone of the carbonyl, whereas in the *epi*-cedrene system the methyl protons enter the shielding zone. Comparative ^{13}C-nmr data reveals that, for the 2-*epi*-cedrene, the C-2 at δ 33.3 is highly shielded compared to its counterpart in the cedrene system (δ_C 44.1) (Table 1).

Our studies on collections of *E. georgei* taken from different locations in the western central regions of Western Australia have confirmed the highly variable nature of this species. This variability is reflected superficially in the appearance of the leaf and sepal structures as well as in the type of terpenoid metabolites isolated from the leaf resins. Varieties of this species have been shown to produce cembrane (7), eremane (E.L. Ghisalberti and P.R. Jefferies, unpublished results), and cedrane diterpenes (4), and *ent*-zizaene sesquiterpenes (8) as major metabolites.

We also had the opportunity of examining another *Eremophila* species, collected near the *E. georgei* sample and tentatively regarded as belonging to the *Eremophila gilesii* complex. Fractionation and separation of the base-soluble portion of the resin extract yielded the hydroxy acid **1** and a mixture of two other acids. This mixture was resolved after methylation to give the bisabolene iso-

TABLE 1. ^{13}C-nmr Spectral Data of Selected Compounds.[a]

Carbon	Compound					
	2[b]	3	7	4	Cedrene[c]	6[c]
C-1	54.5	54.6	67.8	53.3	53.8	65.8
C-2	51.6	41.6	33.3	49.8	41.6	44.1
C-3	29.7	35.9	38.2	30.9	36.2	39.6
C-4	23.2	23.3	23.9	23.4	24.9	26.7
C-5	60.6	60.6	56.8	61.4	59.0	59.3
C-6	51.2	51.5	46.9	51.1	48.0	42.6
C-7	48.2	48.2	51.8	48.4	55.0	58.4
C-8	140.3	141.0	167.4	140.9	140.1	165.4
C-9	120.2	120.4	125.1	120.1	119.0	125.8
C-10	42.1	41.7	204.0	42.8	38.9	203.8
C-11	34.8	35.1	37.2	35.8	40.7	44.4
C-12	34.1	33.9	34.9	33.9	25.7	25.2[d]
C-13	24.6	22.9	23.0	22.8		
C-14	143.0	40.4	40.1	40.2		
C-15	127.2	28.1	28.1	28.1		
C-16	180.2	22.8	22.7	22.8		
C-17	12.4	22.8	22.7	22.8		
C-18	24.0[d]	24.4[d]	25.1[d]	24.2[d]	27.7	28.0[d]
C-19	24.6[d]	24.6[d]	25.5[d]	24.6[d]	24.8	27.2[d]
C-20	168.7	13.8	14.4	64.6	15.5	15.3

[a]$CDCl_3$; 20.1 MHz, except for **2** (75 MHz).

[b]Methoxyl carbon at δ 51.8.

[c]For ease of comparison, the numbering system for the diterpenes has been used for the cedrene derivatives.

[d]Values in any one column may be interchanged.

prenologues **8** and **9**, previously found in *Eremophila foliosissima* (1). Botanically, *E. foliosissima* and *E. gilesii* are regarded as being closely related (9). The isolation of the cedrene isoprenologue **1** suggests an affinity between *E. gilesii* and the *E. georgei* complex, in addition to that reported between the complex and *Eremophila clarkei* (9).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Experimental details have been recorded previously (10). The two plant samples were collected 22 km east of Meekatharra in Western Australia. Specimens have been deposited with the Western Australian Herbarium, Department of Agriculture, and duplicates are kept in the Department of Chemistry.

ISOLATION PROCEDURES.—*From* E. georgei.—A detailed description of the extraction from *E. georgei* (specimen number 830903) has been given (4). The less polar fractions obtained from vlc (petroleum ether/CH_2Cl_2, Si gel) of the NaOH-soluble portion of an extract of *E. georgei* (29.7 g) (4) were pooled and rechromatographed to give a crystalline fraction enriched in the acid ester **2** but containing significant amounts of the exocyclic double bond isomer and the corresponding side-chain dihydro derivative (ca. 25%). Repeated fractional crystallization from petroleum ether/EtOAc and from pentane yielded a small amount of **2** (100 mg).

From Eremophila *sp.*—Leaves and branches (53.2 g) from a sample of the plant tentatively regarded as *E. gilesii* (specimen number 830904) were soaked in Me_2CO for 18 h. The extract (7 g) obtained was partitioned into $NaHCO_3$-soluble (1.5 g), NaOH-soluble (2.8 g), and neutral (0.45 g) fractions. The middle fraction was decolorized with charcoal to give an oil (1.25 g) which was subjected to vlc. Elution with 10% EtOAc/CH_2Cl_2 gave fractions (313 mg) containing mainly two compounds. Methylation of this fraction with CH_2N_2 and radial plate chromatography afforded the tetrahydropyran **8** (31 mg) and the tetrahydrofuran **9** (44 mg) identical in all respects to authentic samples (1). The fractions (262 mg) obtained from vlc on elution with 50% EtOAc/CH_2Cl_2 contained one major component which

was obtained pure after preparative tlc. This compound (75 mg) was identical with an authentic sample of **1** (4).

ACID ESTER **2**.—Microcrystalline: mp 76–77.5°; $[\alpha]_D$ −119° (*c* = 0.5, $CHCl_3$); found C 72.79, H 8.85 ($C_{21}H_{30}O_4$ requires C 72.79, H 8.73%); ^1H nmr (300 MHz, $CDCl_3$) δ 6.78 (dq, 1H, *J* = 6.1, 1.3 Hz, H-14), 5.27 (br m, 1H, H-9), 3.75 (s, 3H, OMe), 1.86 (d, 3H, *J* = 1.3 Hz, H_3-17), 1.67 (d, 3H, *J* = 1.5 Hz, H_3-19), 1.04 (s, 3H, H_3-18); ^{13}C nmr see Table 1; ms *m/z* (% rel. int.) 346 (13), 328 (6), 314 (9), 233 (9), 191 (20), 187 (14), 173 (20), 131 (33), 119 (28), 114 (100), 105 (46).

CONVERSION OF **2** TO HYDROCARBON **3**.—A fraction (700 mg) containing **2** (70%) was dissolved in EtOH (10 ml) and stirred under H_2 in the presence of 10% Pd/C (70 mg) at room temperature for 18 h. The product recovered (708 mg) was dissolved in dry Et_2O (20 ml) and treated with $LiAlH_4$ (700 mg) at reflux for 36 h. The diastereomeric mixture of diols (358 mg) was taken up in pyridine and treated with TsCl (700 mg). The ditosylates (684 mg) were reduced with $LiAlH_4$ (1.2 g)/Et_2O by heating under reflux for 18 h. The product recovered (273 mg) was purified by argentation chromatography (15% $AgNO_3$-Si gel, petroleum ether) to give the hydrocarbon **3** (182 mg): oil; $[\alpha]_D$ −92.8°; identical by ^1H-, ^{13}C-nmr and ms with a sample of **3**, $[\alpha]_D$ −96.9°, described previously (4).

DERIVATIVES OF **2**.—A fraction (516 mg) containing **2** (70%) was dissolved in Et_2O (15 ml), the solution was added dropwise to a slurry of $LiAlH_4$ (798 mg) and $AlCl_3$ (934 mg) in dry Et_2O at 0°, and the mixture was stirred for 18 h at room temperature. The product (459 mg) recovered was dissolved in pyridine and treated with Ac_2O (5 ml) for 18 h. The mixture of acetates (563 mg) in EtOH was stirred under H_2 in the presence of 10% Pd/C (100 mg) for 4 h. The product (481 mg) from hydrogenolysis was dissolved in Et_2O and stirred with $LiAlH_4$ (500 mg) for 1 h at room temperature. Usual workup yielded an oil (337 mg) which was purified by preparative tlc and then by argentation chromatography to give the alcohol **4** (111 mg) as an oil: bp (bath) 140°/0.1 mm; $[\alpha]_D$ −84° (*c* = 4.3, $CHCl_3$); found C 82.19, H 11.89 ($C_{20}H_{24}O$ requires C 82.68, H 11.80%); ^1H nmr (80 MHz, $CDCl_3$) δ 5.23 (m, 1H, H-9), 3.65 (m, 2H, AB part of ABX system, J_{AB} = 11.1 Hz, J_{AX} = 7.2 Hz, J_{BX} = 5.3 Hz, H_2-20), 1.68 (d, 3H, *J* = 1.5 Hz, H_3-19), 1.20 (s, 3H, H_3-18), 0.98 (d, 3H, *J* = 6 Hz) and 0.88 (d, 3H, *J* = 6 Hz), H_3-16 and H_3-17); ^{13}C nmr see Table 1; ms *m/z* (% rel. int.) 290 (10), 259 (19), 177 (14), 159 (48), 145 (20), 105 (100). A sample of the alcohol **4** (25 mg) in HOAc was stirred under H_2 in the presence of PtO_2 (2 mg) to yield the dihydro alcohol **5** (25 mg): oil; bp (bath) 145–150°/0.1 mm; $[\alpha]_D$ −24° (*c* = 2.5, $CHCl_3$); ^1H nmr (80 MHz, $CDCl_3$) δ 3.62 (m, 2H, AB part of ABX system, J_{AB} = 11.1 Hz, J_{AX} = 7.2 Hz, J_{BX} = 5.3 Hz, H_2-20), 1.09 (s, 3H, H_3-18), 0.86 (d, 9H, H_3-16, H_3-17, H_3-19); ms *m/z* (% rel. int.) $[M]^+$ 292 (2), 261 (3), 207 (40), 189 (100), 179 (9), 152 (16), 133 (12).

α-CEDRENONE [**6**].—α-Cedrene (500 mg) in C_6H_6 (10 ml) was treated under N_2 with $Cr(CO)_6$ (268 mg), followed by *t*-BuO_2H (90%, 0.7 ml), and the mixture was heated under reflux for 18 h. The cooled solution was filtered, and the filtrate was diluted with Et_2O. The product recovered on vlc gave fractions containing α-cedrenone (80%). These were pooled and chromatographed over alumina (activity 1, neutral) to give pure cedrenone [**6**]: mp 27–29°; $[\alpha]_D$ −103° (*c* = 0.2, $CHCl_3$); found C 82.66, H 10.26 ($C_{15}H_{22}O$ requires C 82.50, H 10.16%); ν max (film) 1669, 1625 cm^{-1}; λ max (MeOH) 230 (log ε 3.95); ^1H nmr (80 MHz, $CDCl_3$) δ 5.65 (br s, 1H, H-9), 2.33 (d, 1H, *J* = 4 Hz, H-7), 1.97 (d, 3H, *J* = 2 Hz, H_3-14), 1.31 (d, 3H, *J* = 6.0 Hz, H_3-15), 1.14 (s, 3H, H_3-13), 1.01 (s, 3H, H_3-12); ^{13}C nmr see Table 1; ms *m/z* (% rel. int.) 218 (82), 189 (9), 176 (88), 175 (74), 161 (100), 147 (36), 136 (54), 121 (78), 105 (25).

DITERPENE ENONE **7**.—A solution of the hydrocarbon **3** (180 mg) in CH_2Cl_2 (10 ml) was treated with a suspension of CrO_3 (200 mg) in CH_2Cl_2 (5 ml) and pyridine (0.5 ml) with stirring. Fresh batches of the oxidizing reagent were added every 24 h until no more starting material was detected (10 days). The recovered oil (223 mg) was chromatographed over alumina (activity 1, neutral). Elution with 90% petroleum ether/CH_2Cl_2 gave fractions containing the enone **8** (24 mg) as a semi-solid oil: ^1H nmr (80 MHz, $CDCl_3$) δ 5.78 (br s, 1H, H-9), 2.56 (d, 1H, *J* = 3 Hz, H-7), 2.52 (m, 1H, H-2), 1.99 (d, 3H, *J* = 1.7 Hz, H_3-19), 1.63 (m, 2H, H_2-11), 0.94 (s, 3H, H_3-18), 0.92 (d, 6H, *J* = 7.4 Hz, H_3-16 and H_3-17), 0.84 (s, 3H, H_3-20); ^{13}C nmr see Table 1; ms *m/z* (% rel. int.) $[M]^+$ 288 (77), 246 (25), 203 (46), 175 (64), 148 (50), 147 (39), 137 (73), 135 (100), 121 (50), 105 (23).

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