DAMMARENEDIOL II ESTERS FROM *CACALIA ATRIPLICIFOLIA* L. SEED OIL

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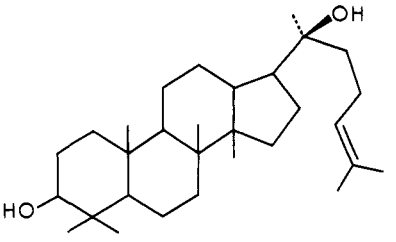
ABSTRACT.—About one-fourth of *Cacalia atriplicifolia* L. seed oil is composed of long-chain fatty esters of dammarenediol II. A minor portion (ca. 2%) is esters of oleanolic acid with long-chain fatty acids, and the remainder of the oil is composed of triglycerides. Dammarenediol II was identified by comparison to authentic material isolated from Gum Damar and by spectroscopy.

In an effort to evaluate plant species for potential industrial exploitation, Buchanan and coworkers (1) singled out *Cacalia atriplicifolia* L. as a potential source of both oil and natural rubber. Since the seeds would supply the major portion of the derived oil, even if whole-plant utilization is practiced, the unusual



Dammarenediol II

1

nature of the seed oil of this species was investigated. Approximately one-fourth of the oil is composed of long-chain fatty esters of dammarenediol II (**1**). A minor amount (ca. 2%) of the oil is made up of long-chain fatty esters of oleanolic acid. Dammarenediol II previously has been reported in resins from certain Dipterocarpaceae (2–5) native to Southeast Asia and in the fruit of *Pouteria caimoto* (6). *C. atriplicifolia* is the first North American species found to contain dammarenediol II and is the only natural source of its long-chain fatty esters.

EXPERIMENTAL

Preliminary fractionation of *C. atriplicifolia* oil was accomplished on a silica column which was eluted sequentially with 150 ml of hexane-ether (95:5), 300 ml of hexane-ether (90:10) and then 300 ml of ether. Terpene esters were eluted with the 90:10 solvent system and were further purified by thin-layer chromatography (tlc) on 1-mm layers of silica with hexane-ether (70:30). For gas chromatography (gc) of intact terpene esters and triglycerides, a 90 ×0.2 cm glass column packed with 3% OV-1 on Gas-Chrom Q was temperature programmed from 200 to 350° at 4°/min. Methyl esters of the fatty acids were identified and quantitated on two gc columns (Apiezon L and LAC-2-R446) (7). For capillary column gc, a 25 m column coated with OV-17 was run at 260°.

The low resolution gas chromatography-mass spectrometry (gs-ms) apparatus and data system has been described previously (8). Molecular formula measurements were made on a Kratos MS-30 dual beam MS with allied data system.

[1]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Proton magnetic resonance (pmr) spectra were obtained from $CDCl_3$ solutions on a Varian XL-100 spectrometer with tetramethylsilane as the internal standard.

Dammarenediols I and II were isolated (as acetates) from Gum Damar (Sigma Chemical Co.) by column chromatography followed by tlc on silica, hexane-ether (60:40) and then high-pressure liquid chromatography (HPLC) on a Magnum-9 ODS-2 column (Whatman, Inc.) with CH_3CN as the mobile phase. They were finally recrystallized from methanol.

Compounds were acetylated in acetic anhydride-pyridine (2:1) and recovered by extraction with ether. They were silylated in pyridine with hexamethyldisilizane-trimethylchlorolsilane (2:1). Acylation of **1** with palmitoyl chloride took place in pyridine. Esters were hydrolyzed in ethanol with 50% aqueous KOH, and the products were recovered by ether extraction after acidification with 6*N* HCl. Methyl esters were prepared by reaction with CH_2N_2 in ether.

RESULTS AND DISCUSSION

The long-chain fatty esters of Dammarenediol II (**1**) migrated about one-half as far as triglycerides on tlc and were eluted immediately before the C_{52} triglycerides by gc. Thus, they were easily separated and monitored chromatographically. Hydrolysis and work-up of the terpene ester fraction followed by esterification of the mixture gave **1**, methyl oleanolate, and the methyl esters of the fatty acids. Further chromatographic isolation and recrystallization from nitromethane yielded **1**, mp 128–130° (uncorr.) $[\alpha]^{25}+31°$ (c 3.34, $CHCl_3$). Literature values are: mp 131–133°, $[\alpha]_D+33°$ (2). For comparison, dammarenediols I and II were isolated from Gum Damar (as acetates). The acetate of **1** had a mp of 134–135° (2) which was not depressed by admixture of dammarenediol II acetate. Their pmr spectra were identical and differ from the spectrum of the epimeric dammarenediol I at the proton signal for the C-20 methyl group. Its chemical shift is found at 1.10 ppm in dammarenediol I and 1.14 ppm in dammarenediol II (for positional numbering of **1**, see reference 9).

Further, the mass spectral characteristics of **1** were studied. It gave no molecular ion (4) but did exhibit a strong ion for $M-18^+$ (20% of base) at *m/e* 426.3930 ($C_{30}H_{50}O$ requires 426.3862). Following silylation, ions could be recognized for $M-15^+$ (*m/e* 573, 0.6%) and $M-90^+$ (498, 26%). Cleavage on either side of the silyloxy group at C-20 gave *m/e* 505 (8%) and 199 (100%).

The palmitate ester of **1** was chromatographically and spectrally identical to the major component among the natural terpene esters. Therefore, the acyl groups must be attached at C-3 since no report of acylation at the hindered C-20 has been found. The hydroxyl group at C-20 will silylate, however, as evidenced by the mass spectrum.

A minor component present in the hydrolysis product of the terpene esters was identified as oleanolic acid by mass spectra and gc retention characteristics of the trimethylsilyl derivative on both packed and capillary columns.

QUANTITATION OF TERPENE ESTERS

An estimation of the amounts of terpene esters was conducted in the following way. A sample of oil was treated exhaustively with CH_2N_2. This reaction caused the esters of oleanolic acid to be more mobile than triglycerides on tlc. The preparation was separated by preparative tlc into three fractions (*viz.* oleanolate esters, triglycerides and dammarenediol esters). Methyl ester compositions determined for the fatty acids liberated from each fraction and for the total oil are given in table 1. A series of simultaneous equations can be written from this data, with each fatty acid percentage as the coefficient of the mole fractions of three types of original esters. For example: % 16:0 total = % 16:0 in triglycerides (Mole Fraction triglycerides) + % 16:0 in dammarenediol esters (M.F. dammarenediol esters) + % 16:0 in oleanolic acid esters (M.F. oleanolic acid esters).

A least squares fit for the 10 fatty acids present in all three fractions gave calculated mole fractions of: triglycerides = 0.76, dammarenediol esters = 0.22 and oleanolic acid esters = 0.02. These values were then applied to the equations to obtain calculated total fatty acid compositions. Comparison of calculated versus observed total compositions gave a standard deviation of 0.5%.

TABLE 1. Fatty acid composition of *C. atriplicifolia* oil by gc (as methyl esters).

Component	Intact oil	Triglycerides	Dammarenediol esters	Oleanolic acid esters
12:0	tr	tr	0.1	0.3
13:0	tr	tr	tr	0.2
14:0	0.1	0.2	3.6	4.8
15:0	0.1	0.1	0.3	1.1
16:0	18.5	5.9	59.5	53.2
16:1	0.2	0.3	0.8	2.3
17:0	0.1	0.2	0.1	0.2
18:0	5.0	1.5	16.1	10.8
18:1	52.0	65.3	5.9	17.7
18:2	19.4	24.5	2.4	4.9
18:3	0.7	0.8	—	—
20:0	2.4	0.3	10.9	4.1
20:1	0.2	0.3	—	—

The data in table 1 also show that the fatty acids are quite specifically oriented in the oil. Terpenoid esters are rich in saturated fatty acids (principally palmitic). On the other hand, the triglycerides have a more "normal" fatty acid profile in that oleic and linoleic acids predominate.

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