In chemotaxonomic. analyses, therefore, it is clearly necessary to examine the same plant part when surveying different species.

EXPERIMENTAL

Plants were grown from seed obtained from the National Vegetable Research Station, Wellesbourne; in a greenhouse under 16 hr day 8 hr night conditions at approximately 20°. Plants to be used were taken at intervals throughout a year and were analysed individually. The plants were divided into leaf laminae (green), leaf bases (white), roots, and rhizomes which were taken to be those parts covered with dried leaf bases [8]. Equal quantities of tissue were taken for analysis from each part of the plant. The amounts varied from plant to plant from 2 g to 5 g. The material was finely chopped up and placed in a 500 ml flask at 40° for 30 min. 500 ml headspace vapour was collected by the method of Freeman and Whenham [3] by a cold trap and introduced into a gas chromatograph. Dual 2 m × 3 mm ID glass columns of Carbowax 1540, 8% on DMDS-treated Chromosorb W were used. The injection temperature was held at 150°, and the column temperature at 50° for 10 min then programmed to rise at 2° per min to 130° where it was held for 15 min. N_2 carrier gas was used at a flow rate of 8 ml/minute. A dual F.I.D. system was used and peaks were integrated electronically. Major peaks were identified by their coincidence with marker compounds and by relative retention indices. Proportions of radicals present in the three major disulphides were determined by the method of Saghir *et al.* [4].

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(−)-(*S,S*)-12-HYDROXY-13-OCTADEC-*cis*-9-ENOLIDE, A 14-MEMBERED LACTONE FROM *CREPIS CONYZAEFOLIA* SEED OIL

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Key Word Index—*Crepis conyzaefolia*; Compositae; large-ring lactone; 12,13-dihydroxyoleic acid; GC–MS, ORD.

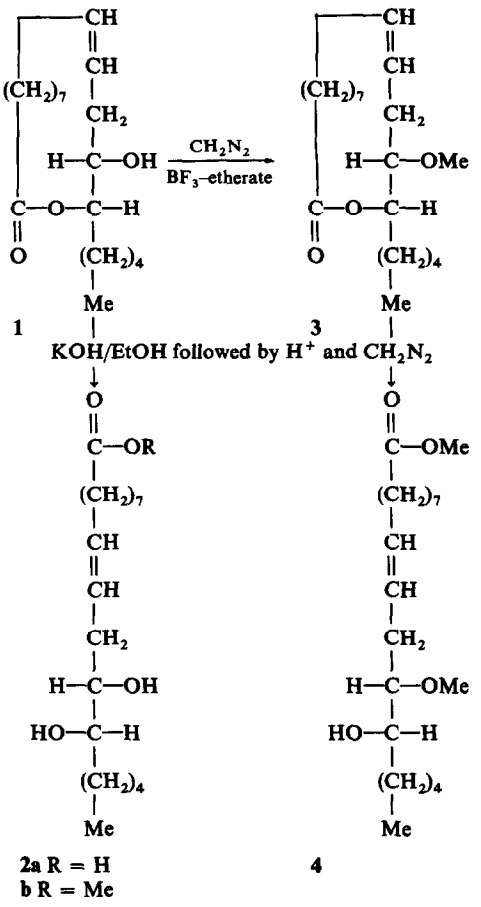
Abstract—*Crepis conyzaefolia* (Gouan) Dalle Torre seed oil contains about 3% of (−)-(*S,S*)-12-hydroxy-13-octadec-*cis*-9-enolide (**1**), a lactone of (−)-*threo*-12,13-dihydroxyoleic acid. The absolute configuration of the acid has been established as D-12, L-13 (12-*S*, 13-*S*) and the lactone has the same absolute configuration.

INTRODUCTION

An earlier investigation of *Crepis conyzaefolia* seed oil disclosed the presence of four epoxy fatty acids—vernolic [(+)-12,13-epoxyoctadec-*cis*-9-enoic], 12,13-epoxystearic, (+)-12,13-epoxyoctadec-*trans*-6-*cis*-9-dienoic and 12,13-epoxy-octadec-*cis*-6-*cis*-9-dienoic [1]. During the isolation of these compounds, a fifth unusual component was collected. It was neither an epoxy acid nor a homolog or isomer of any of the aliphatic acids usually found in seed oils and was not included in the previous fatty acid composition [1]. Its GLC and MS characteristics indicated that it had at least one free hydroxyl group, and the IR spectrum was similar to that of a hydroxy methyl ester (methyl ricinoleate). Its PMR spectrum showed no evidence of methyl ester absorption but otherwise resembled fatty acid spectra. The compound has now been identified as (−)-(*S,S*)-12-hydroxy-13-octadec-*cis*-9-enolide (**1**), a lactone of (−)-*threo*-12,13-dihydroxyoleic acid. It becomes the third large-ring lactone to have been isolated from seed oils [2].

RESULTS

When basic hydrolysis of **1** yielded 12,13-dihydroxyoleic acid (**2a**), a lactone structure was immediately suspected. The lactone obviously contained a free hydroxyl group as indicated by its IR spectrum and MS of its silylation product. Structural proof for the dihydroxyoleic acid was derived from its melting point and ORD values, from its TLC migration and from the



MS of its derivatives. Rigorously purified (−)-*threo*-12,13-dihydroxyoleic acid melts at 61–61.5° and has a specific rotation of −19.0° [3]. Our measurements for **2a** are in good agreement with these values. On boric acid-impregnated TLC plates, *threo*-dihydroxyesters migrate further than their *erythro* isomers [4] and **2b** migrated with the *threo* isomers of 9,10-dihydroxystearate and 12,13-dihydroxystearate rather than the *erythro* diastereomers. The MS of silylated **2b** has major ions at 299 and 173 resulting from cleavage between carbons 12 and 13. A double bond in the 9-position was indicated because double bonds one methylene group removed from silyated hydroxyl groups promote α-cleavage (between C-11 and C-12) [5] giving the intense ion at 275. A 'migration' ion produced by movement of a trimethylsilyl (TMS) group to the ester function [5] is observed at 270. Definitive evidence for placing the double bond at C-9 comes from ozonolysis of **4** (after silyation) which resulted in a 9-carbon aldehyde ester and a 9-carbon methoxy-silyloxyaldehyde.

MS of **1** (after silylation) and of **3** indicated a free hydroxyl group at C-12 and, therefore, the lactone has to be joined at C-13. The spectra had intense molecular ions and no ions from fragmentation between C-12 and C-13 were observed (i.e., no ion at 173). Comparison of the MS of **4** with those of the methoxy-hydroxyoleate isomers derived from vernolic acid clearly shows that **4** is 12-methoxy-13-hydroxyoleate. The double bond one methylene group removed from the oxygenated carbons promotes α-cleavages analogous to those found in the dihydroxy monoene described above. Accordingly, the base peak in the MS of **4** at 145 results from α-cleavage at C-12. Ions from cleavage between the oxygenated carbons occur at 241 and 101 (weak). Other relatively intense ions at 210, 178 and 83 apparently arise from rearrangements and neutral losses of methanol or water from the primary ions 241 and 101. The MS of 12-hydroxy-13-methoxy-oleate is radically different. Although the α-cleavage ion, 145, is prominent, the base peak (115) and the strong ion at 227 are from cleavage between C-12 and C-13 with the expected secondary ions (from 227) found at 209, 195 and 177.

Silylation of **4** gave a product whose MS reinforced the location of the free hydroxyl group since the base peak at 173 results from cleavage between C-12 and C-13 and α-cleavage between C-11 and C-12 gives the ion at 217. The ion containing the methoxy group is less intense than those having the trimethylsilyloxy groups but is nonetheless evident at 241. A trace of dihydroxyoleate can be seen in this spectrum (ions at 299 and 270) which probably resulted from incomplete methylation of **1**.

The (−)-*threo*-isomer of 12,13-dihydroxyoleic acid has been established as D-12, L-13 (12-*S*, 13-*S*) by Morris and Crouchman [6]. In basic hydrolysis of the lactone, configuration of the asymmetric centers is normally retained and, therefore, lactone **1** has the same absolute configuration. Conversion of (+)-vernolic acid, a major constituent of this oil, to the (−)-*threo*-dihydroxyoleic acid is well known [7] but whether or not the lactone is formed totally independent of vernolic acid is not known. If the production of the two is related, the specificity of location and stereochemistry of the lactone indicate that it must be enzymatically derived.

The proportion of the lactone in the oil, as estimated by GC with methyl lignocerate as an internal standard, was 3.0%. Also trace amounts of other lactones containing two double bonds, analogous to the epoxydienoic acids found in this oil [1], were indicated.

EXPERIMENTAL

Instrumental techniques (GC, GC–MS, PMR, IR, HPLC) were applied as previously described [1]. ORD measurements were obtained from ethanolic solutions. Column chromatography of *C. conyzaefolia* oil (1.15 g) was carried out on 20 g of Hi-Flosil with the following solvents: hexane–Et_2O (9:1) 24–15 ml fractions; hexane–Et_2O (3:1) 7–40 ml fractions; hexane–Et_2O (1:1) 10–30 ml fractions. The column was monitored by TLC on Si gel G in hexane–Et_2O (3:1). Ozonolysis was carried out in CH_2Cl_2. Ozonides were reduced with triphenylphosphine prior to GC and GC–MS [8]. Samples were silylated in $CHCl_3$ or CH_2Cl_2 with bis(trimethylsilyl)triflouroacetamide.

Isolation and properties of lactone **1**. The lactone migrated with monoepoxyacyl triglycerides on TLC (R_f = 0.5) and column chromatography [fractions 6 through 14, hexane–Et_2O (3:1)] and was eluted immediately before them by HPLC on μ-Bondapak C_{18} columns and a MeOH–H_2O (3:1) solvent system. Its IR spectrum (CS_2, 10%) had the features of a hydroxy ester: 3570 (sh), 2900–2950 (sh), 1750 (sh) and other bands associated with long-chain esters but with no indications of *trans* unsaturation at 970 cm^{-1}. Its GC retention characteristics were similar to those of methyl ricinoleate [9]. Mp 49–50° (uncorr.) and gave a plain negative ORD: $[\alpha]_D^{26}$ − 38.0° (*c* 0.584 EtOH), $[\alpha]_{560}$ − 41.8°, $[\alpha]_{520}$ − 49.3°, $[\alpha]_{480}$ − 60.3°, $[\alpha]_{440}$ − 76.4°, $[\alpha]_{400}$ − 99.3°, $[\alpha]_{360}$ − 134°, $[\alpha]_{320}$ − 195°, $[\alpha]_{300}$ − 244°. Its MS (70 eV) gave an M^+ at *m/e* 296 (3% rel. int.), an ion for M − 18^+ at 278 (7), 195 (4), 166 (15), 151 (7), 149 (7), 137 (17), 123 (11), 111 (13), 109 (11), 99 (24), 98 (100), 95 (25), 83 (27), 69 (26), 67 (37), 55

(86), 43 (50) and 41 (79). After silylation the spectrum was: 368, M^+ (100), 353 (5), 339 (5), 313 (4), 257 (17), 239 (4), 225 (3), 202 (54), 187 (27), 185 (13), 155 (27), 129 (27), 123 (11), 117 (17), 98 (76), 81 (23), 75 (43), 73 (78), 67 (26). Its PMR spectrum (100 MHz, $CDCl_3$) δ 0.86 (3H, *t*, C-18), δ 1.2–1.4 (18 H, *m*, C-14 through C-17, C-3 through C-7), δ 2.0–2.5 (6H, *m*, C-2, C-8 and C-11), δ 3.66 (1H, *m*, C-12), δ 4.9 (1H, *m*, C-13), δ 5.3–5.7 (2H, *m*, C-9 and C-10) was consistent with structure **1**; the hydroxyl signal was not identified. A mixture of 25.3 mg of *C. conyzaefolia* oil and 1.22 mg of methyl lignocerate were analyzed by GC. Chromatographic response for the lactone **1** was assumed to be equal to that of methyl lignocerate.

(−)-Threo-12,13-dihydroxyoleic acid from lactone **1**. A 29 mg portion of **1** was refluxed 24 hr in 1 ml of 50% aq. KOH and 10 ml EtOH. The saponification mixture was acidified with 6 N HCl, 50 ml H_2O added and then extracted with Et_2O (4 × 10 ml). Solvent was removed from the combined extracts and recrystallization from cold Et_2O (−18°) yielded 20.5 mg of **2a**, mp 59–60° (uncorr.). The ORD of **2a** was a plain negative curve: $[\alpha]_D^{26}$ − 16.6° (*c* 0.464 EtOH), $[\alpha]_{560}$ − 17.9°, $[\alpha]_{520}$ − 21.9°, $[\alpha]_{480}$ − 26.4°, $[\alpha]_{440}$ − 32.6°, $[\alpha]_{400}$ − 42.8°, $[\alpha]_{360}$ − 57.8°, $[\alpha]_{320}$ − 82.1°, $[\alpha]_{300}$ − 113.3°. Treatment of **2a** with CH_2N_2 gave **2b** which, when silylated had a MS identical to that of silylated methyl-12,13-dihydroxyoleate: 457 (0.9), 442 (1.6), 441 (3), 382 (2), 311 (4), 299 (75), 275 (80), 270 (29), 185 (26), 173 (91), 147 (22), 103 (15), 73 (100). TLC of **2b** and authentic samples of *threo-* and *erythro* isomers of 9,10- and 12,13-dihydroxystearates was carried out on boric acid impregnated Si gel with $CHCl_3$ as the solvent [4].

12-Methoxy-13-hydroxyoleic acid from lactone **1**. A few mg of **1** was dissolved in CH_2Cl_2 and treated with CH_2N_2 and BF_3–etherate in a dry-ice Me_2CO bath [10]. Excess CH_2Cl_2 and CH_2N_2 were evaporated, H_2O was added and extraction with Et_2O gave **3**, MS: 310, M^+ (89), 295 (1), 281 (2), 278 (5), 267 (4), 255 (14), 213 (8), 199 (68), 144 (100), 134 (23), 111 (26), 98 (93), 84 (34), 81 (43), 71 (69), 67 (60), 55 (99), 41 (81). Saponification and esterification of **3** with KOH followed by CH_2N_2 gave **4**, MS: 342, M^+ (1), 311 (6), 279 (4), 241 (29), 210 (26), 209 (6), 178 (7), 145 (100), 135 (10), 99 (10), 95 (54), 83 (16), 81 (22), 71 (40), 69 (25). Siylation of **4** gave the following MS: 414, M^+ (0.2), 399 (0.4), 383 (3), 382 (2), 311 (2), 299 (6), 270 (1), 241 (5), 240 (2), 217 (35), 210 (9), 178 (6), 173 (100), 103 (17), 83 (11), 73 (34). Ozonolysis of this silylated product gave two components, one with retention characteristics and MS of a 9-carbon aldehyde-ester [1,11] and the other with the properties expected from a 9-carbon methoxysilyloxyaldehyde, MS: 245 (0.7), 213 (5), 199 (1), 191 (4), 189 (2), 173 (100), 160 (30), 145 (5), 129 (17), 103 (36), 89 (13), 83 (17), 75 (16), 73 (69), 59 (14), 55 (11).

Methoxy-hydroxyoleates from vernolic acid. Authentic vernolic acid was reacted with BF_3–MeOH to produce the isomeric methoxy-hydroxy derivatives [5,12]. After GC separation, the MS of these compounds were: 12-hydroxy-13-methoxyoleate, MS: 325 (1), 311 (2), 280 (2), 279 (2), 253 (6), 227 (66), 209 (3), 198 (12), 195 (80), 177 (6), 166 (43), 149 (11), 148 (11), 145 (52), 124 (11), 115 (100), 114 (11), 98 (11), 95 (20), 83 (68), 74 (15); 12-methoxy-13-hydroxyoleate, MS: 342, M^+ (2) 311 (2), 279 (2), 241 (15), 210 (11), 209 (3), 178 (9), 145 (100), 135 (10), 99 (4), 95 (41), 83 (8), 81 (5), 71 (12), 69 (9).

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