THE TRITERPENE ESTERS OF *DOLICHOTHELE LONGIMAMMA*

GAYLAND F. SPENCER, KATHLEEN PAYNE-WAHL and REBECCA B. WOLF,

Northern Regional Research Center, Agricultural Research Service, US Department of Agriculture,[1] *Peoria, IL 61604*

and JERRY L. MCLAUGHLIN

Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, IN 47907

ABSTRACT.—The triterpene esters of *Dolichothele longimamma* (DC.) Br. and R. (Cactaceae) were identified and quantified. Fatty acids (primarily saturated) with chain lengths from C_8 to C_{18} were found esterified to the C-3 hydroxyl groups of β-amyrin, methyl oleanolate, maniladiol, erythrodiol, and longispinogenin, all triterpenes of the 12-oleanene series. The major fatty acids were lauric and myristic acids. Finding the triterpenes esterified at each stage of biosynthesis supports the hypothesis that acylation occurs early in the pathway.

The identification and characterization of triterpenes in cactus lipids has long been of interest to natural product chemists (1-3). Recently, Kircher identified the triterpenes in organ pipe cactus, *Stenocereus thurberi* (Engelm.) Buxb., (3) and noted that they were found esterified to fatty acids, but he did not investigate the unhydrolyzed terpene esters. Previous work has described the *Dolichothele* alkaloids (4-7). During the defatting of *D. longimamma* (DC.) Br. and R., a Mexican "peyote" cactus, an unusually high yield (10.5%) of lipids was obtained (6); we now report the natural occurrence of triterpene esters in these *Dolichothele* lipids. The lipids were subjected to column chromatography on silica with increasing concentrations of ethyl ether in hexane to form fractions A-C (figure 1). The major portion of fraction A consisted of esters of methyl oleanolate (**1**) which could be separated into two classes by column chromatography or tlc. After

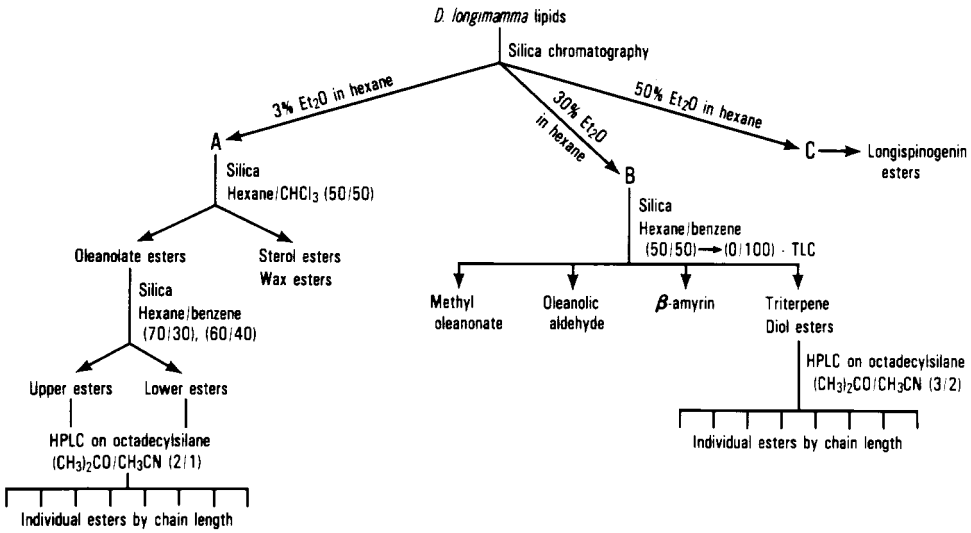
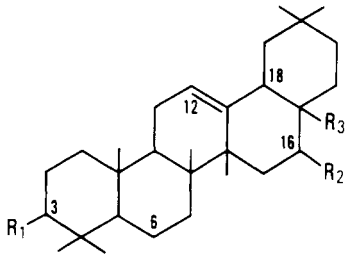


FIGURE 1. Fractionation scheme for *D. longimamma* lipids.

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

repeated chromatography, sufficient purified higher (or faster) and lower (or slower) migrating esters were collected for further fractionation.



1.	R_1 = OH, R_2 = H, R_3 = COOMe	Oleanolate
2.	R_1 ▫ OH, R_2 = H, R_3 = CH_3	β-amyrin
3.	R_1 = O, R_2 ▫ H, R_3 = COOMe	Oleanonate
4.	R_1 = OH, R_2 ▫ H, R_3 = CHO	Oleanolic aldehyde
5.	R_1 = OH, R_2 = OH, R_3 ▫ CH_3	Maniladiol
6.	R_1 = OH, R_2 = H, R_3 = CH_2OH	Erythrodiol
7.	R_1 ▫ OH, R_2 = OH, R_3 = CH_2OH	Longispinoginen

The lower migrating esters were present at levels at least ten times as great as their higher migrating counterparts. Hplc separated each of these two classes according to acyl chain length. The identities of the acyl groups were established after acidic hydrolysis of each fraction followed by gc. The alcohol moiety of the lower migrating esters proved to be methyl oleanolate. Traces of diols and of a dienoate analog of methyl oleanolate [*e.g.*, an isomer of methyl vanquerolate (8)] were indicated among these esters by gc ms. These latter compounds appeared to be even more prominent in the upper migrating esters, but the major compound still had gc and gc ms characteristics identical to those of methyl oleanolate. The reason for the elution pattern of these more lipophilic esters remains unknown, although they may differ in stereochemistry or conformation from those migrating lower. The diols found in trace levels would have to have been completely acylated to occur in these fractions.

A small proportion of fraction A, separated by further column chromatography, was composed of wax esters, sterol esters, and monofunctional triterpene esters. Alcohols identified (after hydrolysis and silylation) by capillary gc and gc ms included octadecan-1-ol, eicosan-1-ol, docosan-1-ol, tetracosan-1-ol, hexacosan-1-ol, octacosan-1-ol, campesterol, stigmasterol, β-sitosterol, and β-amyrin (**2**). These compounds were not isolated by chain length or by class, and the stereochemistry of the sterols was not established. β-Amyrin was clearly the predominant alcohol, followed by the sterols and then the primary alcohols. The overall fatty acid composition of this group of esters is given in table 1.

Further chromatography of fraction B (figure 1) led to the isolation of methyl oleanonate (**3**), oleanolic aldehyde (**4**), methyl oleanolate, and β-amyrin and to a fraction containing the diol esters; free sterols, if present, were not detected. Separation of

TABLE 1. Fatty acid composition of esters from *D. longimamma* lipids (by gc of methyl esters).

Acyl group	Fraction A[a]			Fraction B[a]		Fraction C[a]
	Oleanolate esters		Sterol and wax esters	Diol esters		Triol esters
	upper[b]	lower[b]		erythrodiol	maniladiol	
8:0	tr	4	tr	3	4	2
10:0	7	18	4	15	19	13
12:0	20	28	21	27	31	28
14:0	42	35	31	36	31	30
16:0	26	13	25	16	12	15
18:0	4	1	5	1	tr	3
18:1	—	—	4	—	—	2
18:2	—	—	9	—	—	4

[a]See figure 1.

[b]From tlc on silica gel with benzene-hexane (7:3).

the diol esters, according to alcohol moiety and acyl chain length, was necessary so that the location of acylation could be determined by ms. Identities of the two diols were established from the fully hydrolyzed sample of lipid.

Mass spectra of the two types of diol myristates are shown in figure 2. The major ms fragment from the 12-oleanenes results from retro-Diels-Alder (rda) fragmentation (9), giving the intense ion at *m/z* 234. The maniladiol (**5**) ester (base peak at *m/z* 234) did not undergo nearly as much further fragmentation as the erythrodiol (**6**) ester, where losses of 18 (H_2O) and 31 (CH_3O) from the rda fragment gave ions at *m/z* 216 and 203

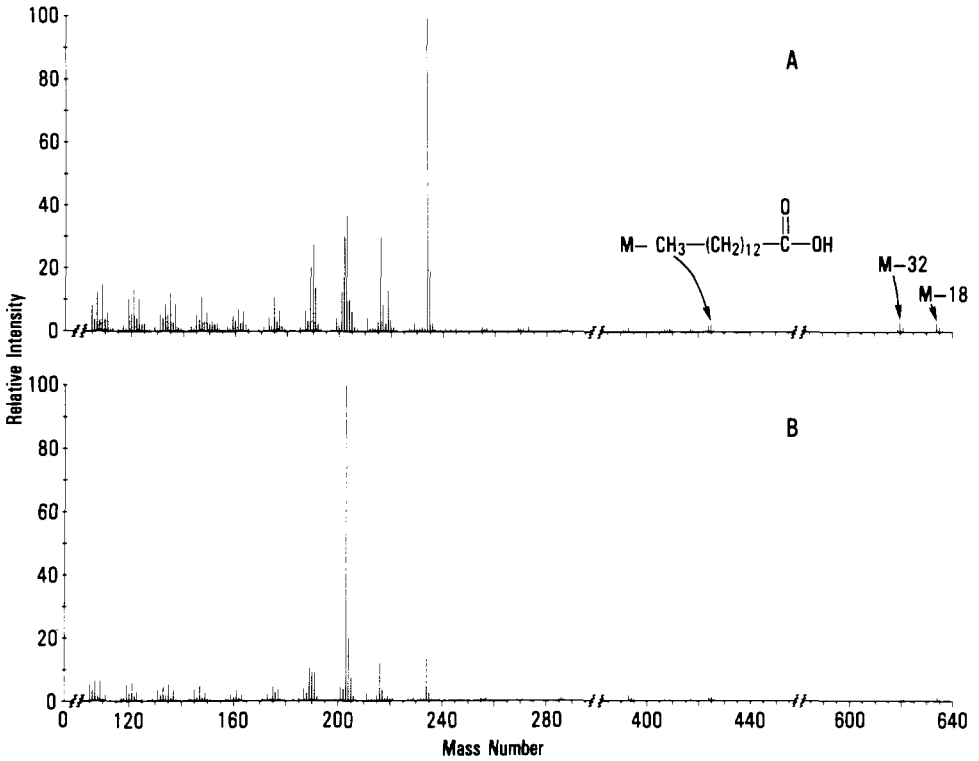


FIGURE 2. Ms of triterpene diol esters. A = maniladiol myristate; B = erythrodiol myristate.

(base peak). These spectra alone indicate that the acyl group must be located at C-3, since rda fragmentation would have yielded an ion at *m/z* 444 if it were located at C-28 (erythrodiol) or C-16 (maniladiol). To determine the validity of this reasoning, trifluoroacetyl (TFA) derivatives of the two myristate esters were prepared and analyzed. These spectra are shown in figure 3. In these spectra, the rda ion appears at *m/z* 330 and is the base peak from the erythrodiol TFA derivative. The maniladiol derivative easily loses trifluoroacetic acid from the rda fragment giving the base peak at *m/z* 216. (This is in contrast to the underivatized ester, which tended to retain the C-16 hydroxy group with the rda fragment.) Compositions of the fatty acids esterified to the two diols are given in table 1.

Longispinogenin (**7**) was the only triterpene triol found in fraction C. The proportions of the fatty acids esterified to it are given in table 1. The mass spectrum of the TFA derivative of the laurate (figure 4) had an intense ion at *m/z* 328, which was formed from loss of trifluoroacetic acid from the rda fragment (426-98). The intact rda ion was not observed with longispinogenin. Further degradation led to the base peak (*m/z* 190). That the rda fragment was unstable is not surprising inasmuch as the TFA derivative of maniladiol readily lost trifluoracetic acid whereas the derivative of erythrodiol retained

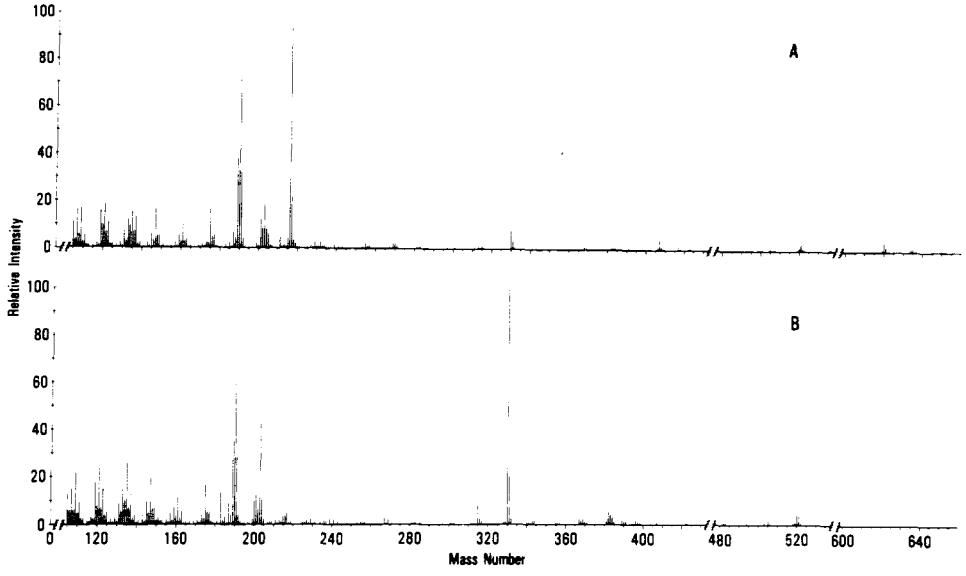


FIGURE 3. Ms of trifluoroacetyl derivatives of triterpene diol esters. A = maniladiol myristate derivative; B = erythrodiol myristate derivative.

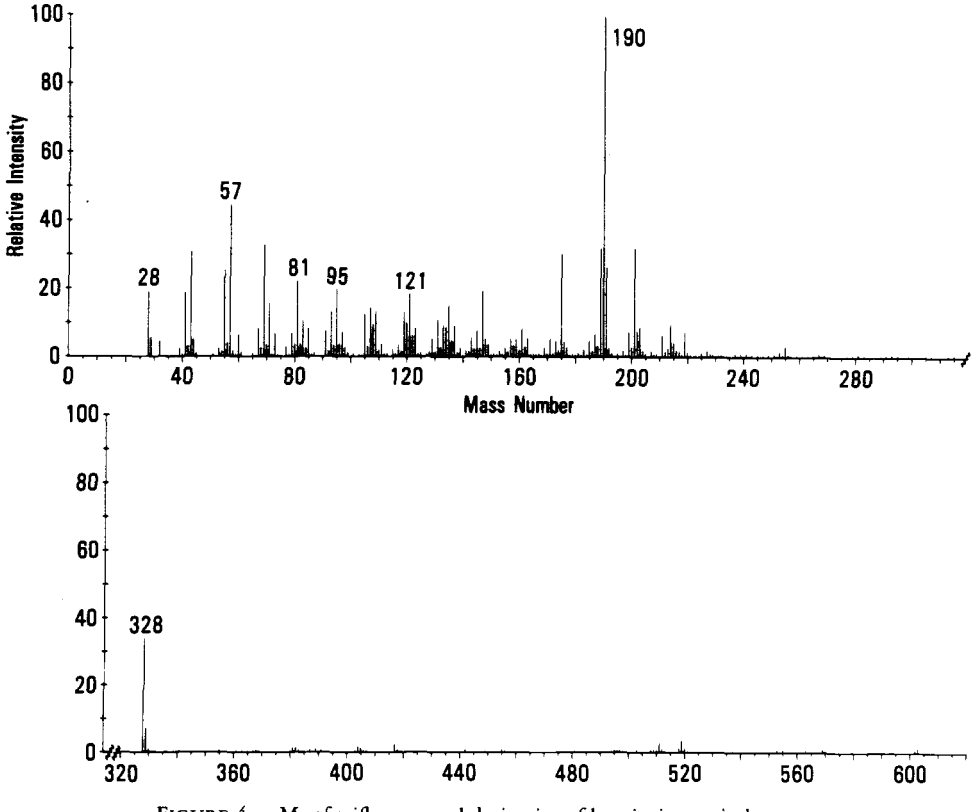
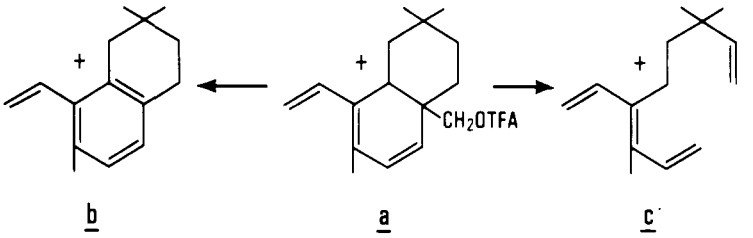


FIGURE 4. Ms of trifluoroacetyl derivative of longispinogenin laurate.

the TFA group. The ion at *m/z* 328 could be expected to have the structure **a** which could decompose to ion **b** (*m/z* 201) or with the loss of C-17 and hydrogen transfer (9) to an ion such as **c** (*m/z* 190).



b a c

Quantitative estimation of the triterpene esters was not feasible by gc because there was overlap among the oleonolate, diol, and triol esters. One reasonable approach was to use hplc with an infrared detector since all of the prominent species contained at least one carbonyl group, a strong infrared chromophore. A slight modification of the method of Payne-Wahl and coworkers (10) gave the separation depicted in figure 5. Tripalmitin was chosen as the internal standard because of its retention position.

Because insufficient reference material was available to determine responses for each type of material present, responses were assumed to be proportional to the number of carbonyl groups per molecule. [This assumption is not exact but can give reasonable results (10).] An average molecular weight could be calculated for the esters (from data in table 1), enabling a check on the material balance. (Compounds without carbonyl groups would not have been detected). Table 2 gives estimations of the compositions of *D. longimamma* lipids obtained by this technique and the composition of similar lipids from *D. uberiformis* (Zucc.) Br. and R. (7). The internal standard allowed estimation of the amounts of materials detected. The data show that over 90% of the amounts injected was recovered.

TABLE 2. Estimated composition of lipids by hplc.

	Sample (mg/ml)[a]			
	D. longimamma (7.3)[a]		*D. uberiformis* (6.5)[a]	
	mM/ml[b]	mg/ml[b]	mM/ml[b]	mg/ml[b]
Sterol and wax esters	0.004	0.24	0.005	0.33
Oleanolate esters	0.049	3.30	0.073	4.80
Methyl oleanonate				
Triterpene diol esters	0.032	2.10	0.018	1.36
Methyl olenolate	0.006	0.27	0.001	0.04
Oleanolic aldehyde	0.004	0.20	0.001	0.05
Triterpene triol esters	0.006	0.40	0.004	0.24
Total		6.51		6.81
Material balance		89%		105%

[a]Concentration of solution prepared for analysis. Two injections (10 and 30 µl) were necessary to estimate both large and small peaks (10).

[b]Concentrations found by hplc. Molar responses were converted to weight through the calculation of an average molecular weight for acylated triterpenes.

The lipid patterns found in *Dolichothele* are clearly similar to those found by Kircher in organ pipe cactus (1) and support his biosynthesis scheme. Whereas he did not find β-amyrin, erythrodiol, or maniladiol, he included them as precursors of oleanolic al-

dehyde and acid. We have found the triterpene constituents to be acylated in all but one instance (oleanolic aldehyde esters were not found; oleanonate will not acylate), suggesting that the acylation must take place early in biosynthesis. This hypothesis is supported by the specificity found among the diol esters where acylation had occurred only at C-3, with C-28 and C-16 bearing free hydroxyls. Unlike organ pipe cactus (1), *Dolichothele* showed no evidence of any triterpene ring system other than oleanene. Triterpenes and alkaloids are not mutually exclusive in *Dolichothele,* unlike certain columnar Mexican cacti (11).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The chromatographic and spectrometric equipment has been described in previous papers (12,13). Mass spectra were generated by electron impact ionization. Hiflosil (Anspec Co., Ann Arbor, MI) was used for silica column chromatography, precoated plates (E. Merck, Darmstadt, BRD) for tlc, ODS-2 (Whatman, Clifton, NJ) for reverse-phase hplc, and Partisil PAC (Whatman) for conventional phase hplc. Triterpenes, triterpene esters, and fatty acid methyl esters were analyzed by gc as previously described (12). For capillary column gc, a 50-M fused silica WCOT column coated with OV-1 (Hewlitt-Packard, Avondale, PA) was temperature programmed from 220° to 270° at 1°/min. Samples were acetylated in acetic or trifluoroacetic anhydride-pyridine (2:1) and then recovered by extraction with Et_2O following the addition of H_2O. Hydrolyses/methanolyses were performed in either basic (0.5N KOH in EtOH) or acidic (1% H_2SO_4 in MeOH) conditions with 4-5 h refluxing times. Recoveries were made by Et_2O extraction. Silylations were accomplished in pyridine with trichloromethylsilane-hexamethyldisilazane (1:2).

PLANT MATERIALS AND LIPID EXTRACTIONS.—Living specimens of *D. longimamma* and *D. uberiformis* were obtained, identified, and freeze-dried as previously described (6,7). Pulverized *D. longimamma* (354 g) was continuously extracted in a Soxhlet extractor for 24 h with petroleum ether (boiling point, 30-60°) to remove lipids (37 g, 10.5%). Similarly, *D. uberiformis* (178 g) afforded 18.6 g (10.4% yield) of lipids.

FRACTIONATION OF *D. LONGIMAMMA* LIPIDS.—The fractionation scheme is summarized in figure 1. Gc analysis of methyl esters prepared from the fractions and subfractions provided the fatty acid compositions given in table 1. The first silica chromatographic column was sequentially eluted with 250-ml portions of 3% Et_2O in hexane, 30% Et_2O in hexane, and 50% Et_2O in hexane to produce fractions A-C. A second silica chromatographic step with hexane-$CHCl_3$ separated the wax esters and sterol esters of fraction A from the oleanolate esters. The latter were then re-chromatographed with benzene-hexane to yield two sets (higher and lower Rf's on silica gel tlc with benzene-hexane, 7:3) of oleanolate esters. Finally, hplc on a reverse-phase column [$(CH_3)_2CO$-CH_3CN (2:1)] separated the individual esters by acyl chain length. Hydrolysis of the mixed esters, followed by tlc and crystallization from MeOH, yielded methyl oleanolate, mp, 198-201°, Lit. mp, 201° (14).

Fraction B was re-chromatographed on silica with a step gradient from hexane-benzene (50:50 to 25:75), and then to benzene. Thirty 10-ml fractions were collected from each solvent step. Further purification of components was accomplished by silica gel tlc in hexane-ethyl ether solvent mixtures. Components isolated were: additional methyl oleanolate; oleanolic aldehyde, gc ms identical to auhentic sample; methyl oleanonate, mp, 182-184°, Lit. mp, 184° (15); ms, identical to literature (9); and β-amyrin, gc ms identical to authentic sample from gum elemi. The major portion of fraction B consisted of triterpene diol esters and was further fractionated by reverse-phase hplc [$(CH_3)_2CO$-CH_3CN (3:2)].

Fraction C was composed essentially of triterpene triol esters that were analyzed with no further partition.

ISOLATION OF MANILADIOL AND AMYRINS FROM GUM ELEMI.—A sample of gum elemi (Sigma Chemical Co., St. Louis, MO) was dissolved in $CHCl_3$ and placed on a dry silica column. The column was sequentially eluted with 250-ml portions of 10%, 20%, 30%, and 50% Et_2O in hexane. The two latter fractions were combined, acetylated, and re-chromatographed on silica with 250 ml of 5% Et_2O in hexane to remove polar constituents. The diacetates were purified by reverse-phase hplc (5% H_2O in MeOH) in the recycle mode (16). After four cycles, separation was complete enough to collect the two components. Each diacetate was recrystallized from MeOH: maniladiol diacetate, mp, 197-198° $[\alpha]^{26}D+81°$ (c = 0.6, $CHCl_3$), Lit. mp, 193-194°, $[\alpha]^{20}D+80°$ (17); brein diacetate, mp, 200-201°, $[\alpha]^{26}D+74°$ (c = 1.3, $CHCl_3$), Lit. mp, 197-198°, $[\alpha]^{17}+70°$ (17).

The fraction eluted with 20% Et_2O in hexane was acetylated and separated by reverse-phase hplc [CH_3CN-$(CH_3)_2CO$, (1:1)]. The individual acetates were crystallized from MeOH-$CHCl_3$ (5:1), giving

α-amyrin acetate mp, 222-224°, Lit. mp, 227° (14) and β-amyrin acetate mp, 242-244°, Lit. mp, 241° (14).

QUANTITATIVE ESTIMATION.—The constituents in *Dolichothele* lipids (table 2) were estimated by a modification of the hplc method described by Payne-Wahl *et al.* (10). The Partisil PXS 10/25 PAC (Whatman, Clifton, NJ) column was solvent programmed from 2% solvent B (hexane-$CHCl_3$-CH_3CN, 30:36:34) to 95% B over 25 min. Solvent A was hexane-$CHCl_3$ (2:1) and the flow rate was 2 ml/min. Tripalmitin was the internal standard, and the responses were considered to be proportional to the number(s) of carbonyl groups per molecule, since individual responses for the compounds were not determined.

IDENTIFICATION OF ALCOHOLS.—A completely hydrolyzed portion of *D. longimamma* lipids was separated into six fractions by conventional-phase hplc with hexane-EtOAc (10:3) (see figure 6). Fraction 1, the solvent front, did not appear to contain significant amounts of lipid materials and was not examined further. Fraction 2 was examined by gc ms (after silylation) and by capillary column gc.

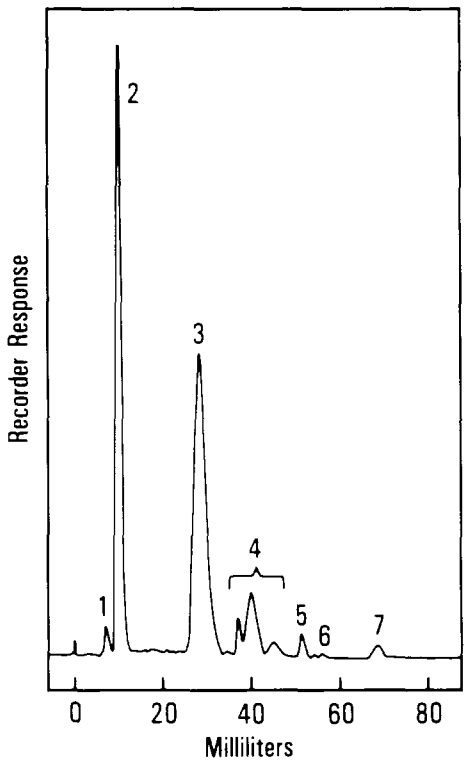


FIGURE 5. Hplc of *D. longimamma* lipids on Partisil PSX 10/25 PAC. Solvents: A = hexane-$CHCl_3$ (2:1), B = hexane-$CHCl_3$-CH_3CN (30:36:34). Solvent programmed from 2% B to 95% B over 25 min. Flow = 2 ml/min. Detector is infrared at 5.75 μ. Peaks: 1 = sterol and wax esters, 2 = oleanolate esters, 3 = tripalmitin (internal standard), 4 = methyl oleanonate and triterpene diol esters, 5 = methyl oleanolate, 6 = oleanolic aldehyde, and 7 = triterpene triol esters.

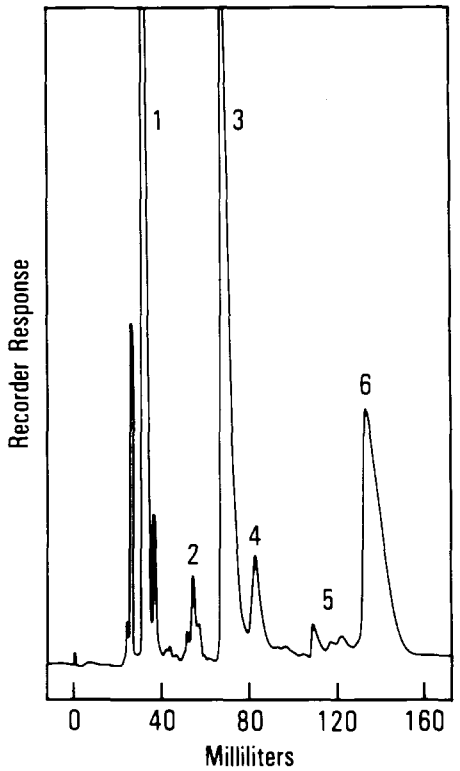


FIGURE 6. Hplc separation of triterpene alcohols from *D. longimamma* lipids. Partisil PSX 10/25 PAC. Solvent: Hexane-EtOAc (10:3). Flow = 5 ml/min. Detection by refractometer. 1 = Solvent front, 2 = primarily β-amyrin, 3 = methyl oleanolate, 4 = primarily phytosterols, 5 = primarily oleanolic aldehyde and methyl oleanonate, 6 = triterpene diols.

Fraction 3 was acetylated and then chromatographed into two components (one major, one minor) by reverse phase hplc with CH_3CN-$(CH_3)_2CO$ (4:1). Both of these were then crystallized from MeOH-$CHCl_3$ (5:1) yielding Ia, acetylmethylolenolate, mp, 219-222°, $[\alpha]^{26}D = +68°$ (c = 2.5 in $CHCl_3$), Lit. mp = 223°, $[\alpha]^{20}D$ +70° (14) as the major component. Not enough of the minor component was available to obtain an optical value, but the mp was 202-205°.

Fraction 4 was purified by another pass through the hplc (removing components of fraction 3) and was examined by gc ms and capillary gc. Fraction 5 was purified by tlc and analyzed by gc ms.

Fraction 6 was acetylated and separated into two components by tlc. Each was crystallized from MeOH giving erythrodiol diacetate, mp, 182-184° $[\alpha]^{26}D$ +59° (c = 9.0 in $CHCl_3$), Lit. mp, 183° $[\alpha]^{20}D$ + 63° (17) and maniladiol diacetate, mp, 196-197°, Lit. mp, 193-194° (17), which was undepressed with admixture of authentic maniladiol diacetate from gum elemi.

Hydrolysis of fraction C (figure 1), acetylation, and purification by reverse-phase hplc [CH_3CN-$(CH_3)_2CO$, (10:1)] gave longispinogenin triacetate mp, 219-221°, Lit. mp, 218° (1), crystallized from MeOH-$CHCl_3$ (10:1).

ACKNOWLEDGMENTS

We wish to thank H.W. Kircher for authentic oleanolic andehyde.

LITERATURE CITED

1. R. Hegnauer, "Chemotaxonomie der Pflanzen," Vol. 3, Birkhäuser Verlag, Basel, 1964, p 324.
2. M Marx, L. Leclercq, B. Tursch, and C. Djerassi, *J. Org. Chem.*, **32**, 3150 (1967).
3. H.W. Kircher, *Phytochemistry*, **19**, 2707 (1980).
4. H. Rosenberg and A.G. Paul, *Phytochemistry*, **9**, 655 (1970).
5. J.J. Dingerdissen and J.L. McLaughlin, *Lloydia*, **36**, 419 (1973).
6. R.L. Ranieri and J.L. McLaughlin, *J. Org. Chem.*, **41**, 319 (1976).
7. R.L. Ranieri and J.L. McLaughlin, *Lloydia*, **40**, 173 (1977).
8. D.M.R. Barton, H.T. Cheung, P.J.L. Daniels, K.G. Lewis, and J.F. McGhie, *J. Chem. Soc.*, 5163 (1962).
9. H. Budzikiewicz, J.M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).
10. K. Payne-Wahl, G.F. Spencer, R.D. Plattner, and R.O. Butterfield, *J. Chromatogr.*, **209**, 61 (1981).
11. A.C. Gibson and K.E. Horak, *Ann. Mo. Bot. Gard.*, **65**, 999 (1978).
12. G.F. Spencer, L.W. Tjarks, and R. Kleiman, *J. Nat. Prod.*, **43**, 724 (1980).
13. G.F. Spencer, *J. Nat. Prod.*, **44**, 166 (1981).
14. M. Windholz, "The Merck Index," 9th ed., Merck and Co., Rahway, 1976, pp 657, 6624.
15. S. Huneck, *Tetrahedron*, **19**, 479 (1963).
16. C.E. Colson and J.M. Lowenstein, "Methods in Enzymology," Vol. 72, Academic press, New York, 1981, Chapter 6.
17. I.M. Morice and J.C.E. Simpson, *J. Chem. Soc.*, 795 (1940).

Received 13 October 1982