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SURFACE WAX COMPONENTS IN SPECIES AND HYBRIDS OF *CORTADERIA*[1]

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Abstract—Analyses of the alkanes and triterpene methyl ethers present in the surface waxes of a New Zealand indigenous species of *Cortaderia* (Gramineae, as yet formally undescribed), of *C. araucana* from South America, and of F_1 interspecific hybrids involving *C. fulvida*, *C. richardii*, and *C. toetoe* are reported. Alkane distribution patterns show, as reported previously, a distinction between New Zealand and South American species in that the latter show a higher ratio of C_{31} *n*-alkanes to C_{29} *n*-alkanes than do New Zealand species. F_1 interspecific hybrids have the characteristic New Zealand pattern. The results of our further triterpene methyl ether analyses reveal that these compounds do occur in *Cortaderia* from South America, and that New Zealand species may lack them; this in contradiction of our earlier findings. F_1 hybrids among New Zealand species produce arundoin; the biosynthesis of the methyl ethers of α- and β-amyrin is suppressed in F_1 *C. richardii* × *C. toetoe*. Cylindrin (isoarborinol methyl ether) is recorded for the first time in *Cortaderia*, in *C. araucana* where it co-occurs with arundoin.

INTRODUCTION

THE CHEMICAL constituents of the surface waxes of several species of *Cortaderia* (Gramineae) from New Zealand and South America were reported in an earlier paper.[1] In particular, GLC analysis of the total alkane fractions from the surface waxes (the 'fingerprinting' technique[2]) showed that the South American species possessed a higher ratio of C_{31} *n*-alkane to C_{29} *n*-alkane than those species native to New Zealand. The South American species investigated at that time were also found to lack triperpene methyl ethers, compounds which were present in three of the New Zealand endemics. Thus by employing two separate chemotaxonomic approaches, namely study of variation in the relative compositions of the homologous series of *n*-alkanes that appear to be of universal occurrence in plant waxes, and the establishment, by inference, of the intracellular presence or absence of specialized enzyme systems responsible for the elaboration of specific, highly individualistic secondary metabolites, there appeared to be clear chemical distinctions between South American and New Zealand species.

At the same time we reported[1] that what was then considered to be a variant of the New Zealand species *Cortaderia toetoe* Zotov, collected at Raglan, west coast North Island,

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[1] This is Part II of a series on *Cortaderia*. For Part I see M. MARTIN-SMITH, G. SUBRAMANIAN and H. E. CONNOR, *Phytochem.* **6**, 559 (1967).

[2] G. EGLINTON, R. J. HAMILTON and M. MARTIN-SMITH, *Phytochem.* **1**, 137 (1962); G. EGLINTON, A. G. GONZALES, R. J. HAMILTON and R. A. RAPHAEL, *Nature* **193**, 739 (1962); G. EGLINTON, A. G. GONZALES, R. J. HAMILTON and R. A. RAPHAEL, *Phytochem.* **1**, 89 (1962); G. EGLINTON and R. J. HAMILTON, in *Chemical Plant Taxonomy* (edited by T. SWAIN), pp. 187–217. Academic Press, New York (1963).

New Zealand, while containing the high proportion of C_{29} *n*-alkane apparently typical of New Zealand species, lacked triterpene methyl ethers. Further field, laboratory and cultural evidence now shows that the material then referred to as Raglan *C. toetoe*[1] is in fact of a species, as yet formally undescribed, with a widespread distribution on the west coast of North Island. Hence further investigation seemed necessary to determine the validity of the chemotaxonomic distinctions previously described. It also seemed useful to attempt preliminary definition of the genetic control of enzyme systems responsible for the elaboration of triterpene methyl ethers using interspecific hybrids. Results from these further studies form the substance of the present paper.

The newly available materials with which the further work was conducted were as follows:

Cortaderia "Raglan", collected from Muriwai, west coast North Island, Auckland Province;
Cortaderia "Chathams", raised from seed collected on Chatham Islands (G*9305);
C. araucana Stapf, raised from seed gathered in Chile (G 7162);
F_1 *C. richardii* (Endl.) Zotov ♀ × *C. fulvida* (Buch.) Zotov ♂, artificially produced hybrid (G 5001);
F_1 *C. fulvida* ♀ × *C. richardii* ♂, artificially produced hybrid (G 6946);
F_1 *C. richardii* ♀ × *C. toetoe* Zotov ♂, artificially produced hybrid (G 7541);
F_1 *C. richardii* ♀ × *Cortaderia* "Raglan" ♂, artificially produced hybrid (G 8917).

We shall, in this paper, refer to plants of the formally undescribed west coast North Island type as *Cortaderia* "Raglan", thus preserving continuity with our earlier paper.[1] Plants from Chatham Islands (lat. 44°S, long. 176°W) some 500 miles east of Christchurch will be referred to as *Cortaderia* "Chathams".

The collection of most plant material and extraction of total surface waxes was carried out at Lincoln in May 1969 in the course of a visit by one of us (M.M.-S.) to New Zealand. Shoots of *Cortaderia* "Raglan" harvested in August, 1967, at Muriwai, west coast North Island, were airmailed to Glasgow. F_1 *C. richardii* × *Cortaderia* "Raglan" was sampled in July 1970 and analysed only for triterpene methyl ethers at Botany Division D.S.I.R.

In view of established variation in the composition of the surface wax on different parts of the plant,[1] care was taken to ensure that the same portions of leaf blades and sheaths were used in the present investigation as had been used earlier. The methods of analysis of the surface waxes were exactly those used previously. Leaves of each freshly harvested sample were cut into 25 cm lengths and immersed in redistilled light petroleum (b.p. 40–60°) for 16 hr at room temp. Pale yellow waxes were obtained by decantation and removal of the solvent under reduced pressure on a rotary film evaporator. The hydrocarbon fraction from each wax sample, obtained after removal of carbonyl compounds through their conversion into dinitrophenylhydrazones, and saponification of the esters present, followed by chromatography over basic alumina, was then subjected to GLC at 225° employing 0·5% Apiezon L supported on Chromosorb W as the stationary phase.

The presence or absence of triterpene methyl ethers was checked initially by a study of the IR spectrum of the crude total surface wax fraction, when the appearance of character-

* G Number is the Botany Division garden's reference number. All material so noted was grown in the experimental gardens of the Botany Division, Department of Scientific and Industrial Research at Lincoln, Canterbury, New Zealand.

istic absorption at 1104 cm^{-1} revealed the presence of compounds of this class.[3] However, in case the concentration of triterpene methyl ethers was too low to permit recognition in this way, all crude waxes were worked up as if they did contains triterpene methyl ethers by chromatography over neutral alumina, and only when no IR absorption at 1104 cm^{-1} could be detected in any of the fractions eluted prior to the ester fractions was it concluded that triterpene methyl ethers were definitely absent. Identification of individual triterpene methyl ethers was then achieved by a combination of GLC and mass spectrometry taking advantage of the established retention times and mass spectral fragmentations of these compounds.[3]

RESULTS

Alkane Distribution Pattern

The *n*-alkane distribution patterns on leaves of the various samples of *Cortaderia*, determined by GLC analysis, are shown in Table 1. From the results it is immediately apparent that the C_{29} *n*-alkane is the major alkane present in New Zealand *Cortaderia* "Raglan", *Cortaderia* "Chathams" and in the three F_1 hybrids while the C_{31} *n*-alkane is the predominant alkane in the South American *C. araucana*. This is in agreement with our earlier generalization that South American species have a higher ratio of C_{31} *n*-alkane to C_{29} *n*-alkane than do New Zealand species.

Comparison of the results obtained with *Cortaderia* "Raglan" from Muriwai and those previously reported for plants of the same type from the beach at Raglan shows some variation, which is probably seasonal. In particular the present work showed the presence in *Cortaderia* "Raglan" of appreciable percentages of C_{19}, C_{20} and C_{21} *n*-alkanes, which were not detected in the earlier work, and of somewhat lower percentages of the C_{23} *n*-alkane (2% as against 5%), C_{25} *n*-alkane (2% as against 6%), C_{27} *n*-alkane (3% as against 5%), C_{30} *n*-alkane (3% as against 7%) and C_{31} *n*-alkane (5% as against 13%) than were found in the earlier studies.[1]

The three F_1 hybrids among New Zealand species show essentially similar alkane distribution patterns to each other and also to the patterns found for the individual species from which the hybrids were made. For ease of comparison the earlier results from Lincoln garden-grown *C. toetoe*, *C. fulvida* and *C. richardii* are given in Table 2. Comparison of Tables 1 and 2 immediately reveals that a lower absolute concentration of the major C_{29} *n*-alkane was found in the present work. This is accounted for by the higher concentrations of the C_{19} to C_{27} *n*-alkanes.

In contrast to the earlier work,[1] evidence was found from the GLC experiments of the presence of traces of branched chain alkanes. However, in no case were these greater than 1% of the entire alkane fraction.

Triterpene Methyl Ethers

No triterpene methyl ethers were found in *Cortaderia* "Raglan" collected from Muriwai, confirming the earlier report for material of this type collected at Raglan Beach, thus invalidating the earlier generalization that New Zealand species of *Cortaderia* elaborate triterpene methyl ethers while South American species do not. Further evidence as to the lack of validity of this postulate was forthcoming when it was similarly discovered that *Cortaderia* "Chathams" did not produce triterpene methyl ethers either, and that Chilean *C. araucana* contained two triterpene methyl ethers. These were identified as arundoin

[3] T. A. BRYCE, M. MARTIN-SMITH, G. OSSKE, K. SCHREIBER and G. SUBRAMANIAN, *Tetrahedron* **23**, 1283 (1967).

TABLE 1. PERCENTAGE COMPOSITION OF SURFACE ALKANES FROM THE WAXES OF THE LEAVES OF *Cortaderia* AS DETERMINED BY GLC ON AN 0·5% APIEZON "L" COLUMN AT 225°

Plant material	Mole percentage of *n*-Alkane defined by carbon atom content*†												
	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}
Cortaderia "Raglan" (Muriwai); August, 1967	1	1	2	+	2	1	2	1	3	2	72	3	5
Cortaderia "Chathams" (Lincoln); May, 1969	+	1	1	1	2	1	2	2	4	4	71	4	6
F_1 *C. richardii* × *C fulvida* (Lincoln); May, 1969	1	2	3	2	2	3	3	3	5	9	56	4	6
F_1 *C. fulvida* × *C. richardii* (Lincoln); May, 1969	+	+	1	1	1	1	2	2	4	4	68	4	11
F_1 *C. richardii* × *C toetoe* (Lincoln); May, 1969	+	1	1	2	3	3	4	4	5	8	58	3	6
C. araucana (Lincoln); May, 1969	+	1	1	1	2	2	3	3	3	3	35	3	40

and cylindrin respectively on the grounds of GLC retention time and mass spectral fragmentation patterns.

Importantly, the hybrids F_1 *C. richardii* × *C. fulvida*, its reciprocal, F_1 *C. richardii* × *C. toetoe* and F_1 *C. richardii* × *Cortaderia* "Raglan" all contained but one triterpene methyl ether–arundoin (GLC retention time, mass spectral fragmentation and absence of a mixed melting point depression). In *C. richardii* × *C. toetoe* α-amyrin methyl ether and β-amyrin methyl ether were absent.

An investigation of the more polar fraction extracted with chloroform from the surface of the leaves of *Cortaderia* "Raglan" failed to reveal the presence of any triterpene alcohols which could indicate that it was not absence of the methylation step converting the triterpene alcohol (initially formed from the cyclization of squalene epoxide) into the methyl ether that was responsible for the absence of triterpene methyl ethers in these plants. In this

TABLE 2. PERCENTAGE COMPOSITION OF SURFACE ALKANES FROM THE LEAVES OF *Cortaderia* AS DETERMINED BY GLC ON AN 0·5% APIEZON "L" COLUMN AT 240° AS REPORTED IN PREVIOUS WORK[1]

Plant material	Mole percentage of *n*-Alkane defined by carbon atom content*‡												
	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}
Cortaderia "Raglan"; March, 1965§				+	5	1	6	2	5	3	58	7	13
C. richardii (Lincoln); July, 1965				+	+	+	+	+	2	2	86	2	6
C. fulvida (Lincoln); July, 1965							+	+	3	9	80	2	4
C. toetoe (Lincoln); July 1965				+	+	+	3	+	5	2	83	2	+

* The content of an individual alkane is expressed as a mole percentage of the total hydrocarbon content from C_{19}–C_{31} inclusive. The mole percentage is taken as being equivalent to the area percentage, i.e. $100\ A_n/\Sigma_{19}^{31}A_n$, where A_n is the area of the peak corresponding to the hydrocarbon C_n $H_{(2n+2)}$ as measured by planimeter. The values are approximated to the nearest 1 per cent and peaks of relative area less than 1 per cent are indicated by +.

† Compounds identified by co-chromatography (C_{22}, C_{24} and C_{28}) and by log retention times.

‡ Compounds identified by co-chromatography (C_{29} and C_{31}) and by log retention times.

§ Reported under the name Raglan *Cortaderia toetoe*.

connection it should be noted that fernenol, the triterpene alcohol corresponding to arundoin has been shown to co-occur with arundoin in the rhizomes of *Imperata cylindrica*.[4]

In practice it did not prove possible to separate the mixed arundoin and cylindrin isolated from *C. araucana* owing to the small quantities available and to lack of high resolution preparative GLC facilities. The Japanese workers, with greater quantities of material available, separated their mixture of arundoin and cylindrin from *I. cylindrica* by fractional crystallization, when cylindrin crystallized first as the more insoluble of the two.[5] However, the GLC retention times and mass spectral fragmentation patterns of the two components of the mixture from *C. araucana* leave no doubt as to their identity. Thus the retention times of the two components relative to 5α-cholestane on a 0·5% Apiezon L column at 240° were 4·29 and 4·91 respectively (reported[3] for arundoin 4·31 and cylindrin 4·95) while the mass spectral fragmentation corresponded to a combination of those of arundoin and cylindrin, which show identical fragmentation apart from the relative intensities of certain ions.[3] Significantly the main difference between the mass spectrum of the mixed components from *C. araucana* and that of arundoin was an intensified abundance of the ion at $m/e = 355$ as would be expected to be contributed by cylindrin.[3]

DISCUSSION

It is now quite apparent that the presence or absence of triterpene methyl ethers from a particular species of *Cortaderia* is in no way related to a New Zealand or South American origin. However, from the species so far examined there would not seem to be any contradiction of the generalization that South American species show a higher ratio of C_{31} *n*-alkane to C_{29} *n*-alkane than New Zealand species.

The absence of triterpene methyl ethers and the essential similarity in alkane pattern between *Cortaderia* "Raglan" and *Cortaderia* "Chathams" is of interest. Although plants from these two areas do differ in stature, and in panicle, spikelet, floret and floral organ dimensions (the Chatham Is. specimens always being the smaller) nevertheless in general terms these two plants seem to have more in common with each other than with other New Zealand indigenous species. Whatever their relationship, Chatham Is. plants and those from west coast North Island are biochemically separated from other endemic New Zealand species.

The co-occurrence of arundoin and cylindrin in *C. araucana* is an addition to the long list of similar occurrences recently reported in the Gramineae.[6] Arundoin has also been identified in the leaf wax of three species of *Chionochloa*,[7] New Zealand endemics, indicating still further its wide generic distribution in the grasses.

Hybrids between *C. fulvida* and *C. richardii* yielded arundoin, as could be expected because both species synthesize this compound. The hybrid between a species elaborating arundoin and a non-elaborating species (*C. richardii* × *C.* "Raglan") produced arundoin, while the hybrid between arundoin-synthesizing *C. richardii*, and a species synthesizing arundoin and α- and β-amyrin methyl ethers, *C. toetoe*, yielded arundoin only. These results indicate that arundoin synthesis is dominant over non-synthesis, and that synthesis of α- and β-amyrin methyl ethers is recessive to non-synthesis.

[4] K. Nishimoto, M. Ito, S. Natori and T. Ohmoto, *Tetrahedron* **24**, 735 (1968).
[5] K. Nishimoto, M. Ito, S. Natori and T. Ohmoto, *Tetrahedron Letters* 2245 (1965); P. Kanchanapee, K. Nishimoto, T. Kuwamura and S. Natori, *Shoya Kagaku Zasshi* **21**, 65 (1967).
[6] T. Ohmoto, M. Ikuse and S. Natori, *Phytochem.* **9**, 2137 (1970).
[7] H. E. Connor and A. W. Purdie, unpublished results. Arundoin which has been isolated and identified from its GLC retention time and mass spectral cracking pattern is not the sole triterpene methyl ether present in *Chionochloa*, but the other constituents have not yet been fully identified.

The three triterpene methyl ethers in *C. toetoe* have different carbon skeletons although the methyl ethers of β-amyrin (unrearranged oleanane skeleton) and α-amyrin (ursane skeleton) can reasonably be expected to arise from minor variations at the termination of the same main biosynthetic pathway.[8] Arundoin is an E:C-*friedo*isohopane derivative. But irrespective of whether these different nuclear systems result from independent synchronous cyclizations of all *trans* squalene epoxide[9] in different conformational foldings[8] or from different pathways emanating from a common intermediate (compare the tricyclic perhydrocyclopentanonaphthalene carbonium ion proposed by van Tamelen *et al.*[10] for sterol biosynthesis), assuming independent gene–enzyme systems for each individual triterpene methyl ether, F_2 families of *C. richardii* × *C. toetoe* can be expected to show segregation for genes controlling the biosynthesis of the methyl ethers of α- and β-amyrin. It will be necessary to screen a large number of F_2 plants to identify the four classes of recombinants expected. Backcrosses F_1 × *C. richardii* have been made. Flowering time differences have so far prevented the production of the backcross F_1 × *C. toetoe* which would have allowed a smaller screening for α- and β-amyrin methyl ether segregates.

Major differences in time of flowering have prevented pollinations that would produce other crosses between non-elaborators of triterpene methyl ethers and those species elaborating them, e.g. *C. toetoe* (10*x*) × *C.* "Raglan" (10*x*) or *C. araucana* (8*x*) × *C. selloana* (8*x*), thus limiting the genetic evidence. But in *Cortaderia* as at present understood there are genetic systems permitting or preventing the biosynthesis of triterpene methyl ethers. Among the New Zealand species synthesis of arundoin is dominant while α- and β-amyrin methyl ether synthesis is recessive to non-synthesis. For South American species we have no evidence for genetic control but the pattern, where some species elaborate triterpene methyl ethers and others do not, is parallel to that in New Zealand.

EXPERIMENTAL

All m.p.s are corrected. IR spectra were determined on a Perkin–Elmer 157 spectrometer in KCl discs. Mass spectra were determined with an A.E.I.M.S.9. double-focusing mass spectrometer with a solid insertion probe. Spectra were run at 8 kV. The electron beam was 70 eV and the trap current was 100 μA.

The instrument employed in the GLC analysis was a Perkin–Elmer F 11 gas chromatograph fitted with coiled columns 6 ft in length containing 0·5% Apiezon L supported on DCMS treated Chromosorb W of 100–120 mesh, the detector current being fed into a Honeywell Brown recorder. Direct injections (1 μl of a chloroform solution of the compounds under investigation) were made on to the column through a silicone-rubber 'blind hole' stopper. Standard conditions were as follows: column temperature 225 ± 1° (alkane analyses) and 240 ± 1° (triterpene methyl ether analyses), detector temperatures 235 ± 1 and 248 ± 1° respectively; argon flow rate, 45 ml/min, ionization amplifier range, 1 × 10^2.

Isolation of Surface Waxes

This was performed as previously described.[1] The total light petroleum extractives were as follows: *Cortaderia* "Raglan", 6 lb green weight—22 7 g. *Cortaderia* "Chathams" (G 9305), 1 lb 1 oz green weight—3 42 g. F_1 *C richardii* × *C. fulvida* (G 5001), 12 lb green weight—30·7 g. F_1 *C fulvida* × *C. richardii* (G 6946), 6 lb 10 oz green weight—21·2 g. F_1 *C. richardii* × *C. toetoe* (G 7541), 7 lb 8 oz green weight—18·2 g *C. araucana* (G 7162), 2 lb 10 oz green weight—9 2 g.

[8] cf A. ESCHENMOSER, L RUZICKA, O. JEGER and D. ARIGONI, *Helv Chim. Acta* **38,** 1890 (1955); L. RUZICKA, *Proc. Chem. Soc.* 341, (1959); L. RUZICKA, *Pure and Applied Chem.* **6,** 493 (1963); J. H. RICHARDS and J. B. HENDRICKSON, *The Biosynthesis of Steroids, Terpenes and Acetogenins*, pp 257–288 Benjamin, New York (1964).

[9] *Inter alia* E. E. VAN TAMELEN, J. D. WILLETT, R. B. CLAYTON and K. E. LORD, *J. Am. Chem. Soc.* **88,** 4752 (1966); E. J. COREY and W. E. RUSSEY, *J. Am. Chem. Soc.* **88,** 4750 (1966), E. J. COREY and S. K. GROSS, *J. Am. Chem. Soc* **90,** 5045 (1968); W. O. GODTFREDSEN, H LORCK, E. E. VAN TAMELEN, J. D. WILLETT and R. B. CLAYTON, *J. Am. Chem. Soc.* **90,** 208 (1968); H. H. REES, L. J. GOAD and T. W. GOODWIN, *Tetrahedron Letters* 723 (1968), E. E. VAN TAMELEN, *Accounts of Chemical Research* **1,** 111 (1968).

[10] E E. VAN TAMELEN, J. D. WILLETT and R. B. CLAYTON, *J. Am. Chem. Soc.* **89,** 3371 (1967).

Alkane Fractions

These were isolated and subjected to GLC as previously described.[1] Where IR absorption at 1104 cm^{-1} was present in the alkane fraction (with F_1 *C. richardii* × *C. fulvida;* F_1 *C. fulvida* × *C. richardii;* F_1 *C. richardii* × *C. toetoe,* and *C. araucana*) the total alkane fraction was subjected to treatment with conc. H_2SO_4 as previously described[1] before GLC analysis. Identification of the individual *n*-alkanes present in the mixtures was achieved by co-GLC experiments employing authentic *n*-docosane, *n*-tetracosane and *n*-octacosane and plotting log retention time against carbon atom number.

The weights of the total alkane fractions per gram of total light petroleum extractives were as follows: *Cortaderia* "Raglan", 49 mg; F_1 *C. richardii* × *C. fulvida*, 36 mg; *C. fulvida* × *C. richardii*, 39 mg; *C. richardii* × *C. toetoe*, 38 mg; *Cortaderia* "Chathams", 42 mg; and *C. araucana*, 37 mg.

Triterpene Methyl Ethers

Total light petroleum extractives (2·0 g) from each plant were worked up for triterpene methyl ethers as described previously.[1] Crystalline triterpene methyl ether fractions were obtained from F_1 *C. richardii* × *C. fulvida* (32 mg); F_1 *C. fulvida* × *C. richardii* (40 mg); F_1 *C. richardii* × *C. toetoe* (35 mg); and *C. araucana* (42 mg).

The fractions from the three interspecific hybrids all gave a single peak when subjected to the standard GLC conditions (retention time relative to 5α-cholestane = 4·29; reported[3] for arundoin, 4·31). All three specimens had m.p. 236–237° from ethyl acetate, undepressed on admixture with authentic arundoin, and the IR spectra and mass spectra were indistinguishable from those of authentic arundoin.

At Botany Division D.S.I.R., following the methods used at Glasgow, a crystalline triterpene methyl ether fraction was obtained from F_1 *C. richardii* × *C.* "Raglan". MP, IR spectrum, and GLC retention time, were indistinguishable from those of authentic arundoin.

The triterpene methyl ether fraction from *C. araucana* had m.p. 268–270° and gave two peaks under the standard GLC conditions. These had retention times, relative to 5α-cholestane, of 4·29 and 4·91 respectively (reported[3] for arundoin, 4·31 and for cylindrin, 4 95). Admixture first with arundoin and then with cylindrin gave intensification of the appropriate peak. The mass spectral fragmentation pattern was as expected[3] for a mixture of arundoin and cylindrin.

Investigation of the Polar Fraction of the Leaf Surface Wax of Cortaderia "*Raglan*"

Leaves of *Cortaderia* "Raglan" (6 lb) after extraction with light petroleum (b.p. 40–60°) were immersed in $CHCl_3$ for 16 hr. Decantation and removal of solvent gave a solid residue (4 g). After thorough extraction with light petroleum (b.p. 40–60°) the remaining residue was taken up in $CHCl_3$ (100 ml) and the solution extracted with 10% $NaHCO_3$ (5 × 100 ml).

The $CHCl_3$ soluble material (1·2 g) was chromatographed over alumina (Woelm grade 1, 30 g) and the various fractions obtained were subjected to mass spectrometry. The highest molecular weight observed for any of these fractions was *m/e* 326·31750 (calc. for $C_{21}H_{42}O_2^+$, 326·31846) indicating the absence of triterpene alcohols.

Work-up of the combined $NaHCO_3$ solutions and application of mass spectrometry to the organic residue revealed peaks at *m/e* 226·15621 (calc. for $C_{13}H_{22}O_3^+$, 226·15689); *m/e* 211·13305 (calc. for $C_{12}H_{19}O_3^+$, 211·13341); and *m/e* 217·14351 (calc. for $C_{11}H_{21}O_4^+$, 217·14397).

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