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SURFACE WAX COMPONENTS OF FIVE SPECIES OF *CORTADERIA* (GRAMINEAE)—A CHEMOTAXONOMIC COMPARISON

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Abstract—Analyses of the alkanes, fatty acids, *n*-alkanols and triterpene methyl ethers present in the surface waxes of three indigenous New Zealand species of *Cortaderia*—*C. fulvida*, *C. richardii*, *C. toetoe*—and two South American species naturalized in New Zealand—*C. atacamensis*, *C. selloana*—were performed by means of gas–liquid chromatography and the results applied to an attempted chemical identification of *Cortaderia* collected at Raglan and Plimmerton, respectively. Although the alkane distribution patterns of the New Zealand species of *Cortaderia* were similar and so did not permit of any chemotaxonomic distinction among them, there appeared to be a distinction between the New Zealand and South American species in that the former showed a lower percentage of the C_{31} component as compared to the C_{29} component than the latter. No distinction among any of the species was possible from the results of the fatty acid and *n*-alkanol analyses but the triterpene methyl ether analyses showed that only New Zealand species contained compounds of this type. Moreover, *C. toetoe* contained arundoin and the methyl ethers of α-amyrin and β-amyrin whilst *C. fulvida* and *C. richardii* contained arundoin only.

INTRODUCTION

IN AN earlier paper[1] the analysis of the leaf surface alkanes of a grass, at that time designated *Arundo conspicua* Forst. f.,[2] was reported together with evidence for the presence of triterpene methyl ethers, one of which, arundoin,[3,4] is now established[5] as 3β-methoxy-E:C-*friedo*-isohop-9(11)-ene (I). This paper presents more extensive results from examinations of the composition of the leaf surface waxes in five species of *Cortaderia* from New Zealand, and was stimulated by a recent revision of the grass sub-family Arundinoideae,[6] by the absence of arundoin in a sample of *Cortaderia* from Raglan,[7] and because of other detailed botanical studies.[8,9]

[1] G. EGLINTON, R. J. HAMILTON and M. MARTIN-SMITH, *Phytochem.* **1**, 137 (1962).
[2] T. F. CHEESEMAN, *Manual of the New Zealand Flora*, 2nd ed., Government Printer, Wellington (1925).
[3] G. EGLINTON, R. J. HAMILTON, M. MARTIN-SMITH, S. J. SMITH and G. SUBRAMANIAN, *Tetrahedron Letters*, No. 34, 2323 (1964).
[4] T. A. BRYCE, G. EGLINTON, R. J. HAMILTON, M. MARTIN-SMITH and G. SUBRAMANIAN, *Phytochem.* In press.
[5] K. NISHIMOTO, M. ITO, S. NATORI and T. OHMOTO, *Tetrahedron Letters*, No. 27, 2245 (1965).
[6] V. D. ZOTOV, *New Zealand J. Botany* **1**, 78 (1963).
[7] This material was kindly collected and extracted by Drs. R. Hodges and E. P. White of the Animal Research Station, Ruakura, New Zealand, to whom the present authors wish to express their sincere appreciation.
[8] H. E. CONNOR, *New Zealand J. Botany* **1**, 258 (1963); H. E. CONNOR and E. D. PENNY, *New Zealand J. Agri. Res.* **3**, 725 (1960).
[9] H. E. CONNOR, *New Zealand J. Botany* **3**, 17 (1965).

The two New Zealand species formerly regarded as *Arundo* have been transferred to *Cortaderia* Stapf. (*C. fulvida* (Buch.) Zotov and *C. richardii* (Endl.) Zotov) and a new species, *C. toetoe* Zotov, recognized.[6] The individual name *Arundo conspicua* used in the original chemical work[1,3] was the name then current for *Cortaderia richardii* and *C. toetoe*, but was applied in error and the name *A. conspicua* strictly applies to a species of the related genus *Chionochloa*.[6] *Cortaderia* is a genus of about 25 species of which all except the three New Zealand representatives are indigenous to the South American continent. Two of the South American species, *C. atacamensis* (Philippi) Pilger[9] and *C. selloana* (Schult.) Aschers. et Graebn.,[6] have, however, become naturalized in New Zealand.

In the circumstances it seemed desirable to undertake a detailed comparison of the chemical constituents of the surface waxes of each of these five species, but since the surface wax is not uniformly distributed over the leaves of *Cortaderia*, care was taken to ensure that comparable parts of each plant were used as sources of the waxes. In the case of the species indigenous to New Zealand the variation in distribution of the surface wax is easily visible to the naked eye, with an abundant, thick, white, easily scraped covering occurring abaxially on the leaf sheaths, but only very rarely on the lowest part of the leaf blades. By contrast the leaf sheaths in the two naturalized species, though waxy to the touch, bear no such distinctive thick, white layer. Both adaxial and abaxial surfaces of the leaf blades are waxy to the touch in all five species in New Zealand and the slight bloom is easily wiped off.

Surface wax is most abundant on the leaf sheaths of young tillers but even here it wears off readily, mostly from sheaths rubbing together in the wind or from the brushing of the leaf sheaths by nearby blades. On older leaves the sheaths appear less wax-covered.

The sub-microscopic structure of the leaf surface wax has not been investigated.

As a check on variation of surface wax composition between parts of the plant, a comparison was made of the composition of the wax obtained from the leaves with that of the wax obtained from the basal shoots and roots of the abundantly available *Cortaderia* growing in swampy habitat at Plimmerton, Wellington Province, which served as the original source of arundoin.[1,3]

The leaf surface waxes of garden-grown samples of the five species of *Cortaderia* were obtained by extraction with redistilled light petroleum and gas–liquid chromatographic analyses were then performed on the alkane fractions, the derived acetates of the total alkanols and the derived methyl esters of the total fatty acids present in an attempt to secure chemotaxonomic differentiation among them. No distinction was made between alkanols and fatty acids occurring in the free state and alkanols and fatty acids occurring combined as esters. In addition, an investigation was made as to the presence or absence of triterpene methyl ethers in the surface wax of each species, and where compounds of this class were present they were examined for homogeneity by means of gas–liquid chromatography.

Similar analyses were also made of the surface wax constituents of the fresh batch of material collected at Plimmerton and of plant material collected at Raglan in an attempt to determine whether such chemical work would permit of identification at the specific level of these two *Cortaderia* collections. Both the Plimmerton and Raglan material were later identified as *C. toetoe* and will be referred to as such throughout the text but prefixed with the locality names Plimmerton and Raglan.

The use of a combination of total alkane, alkanol and fatty acid analyses together with the determination of the presence or absence of triterpene methyl ethers in the different *Cortaderia* species embraces two separate chemotaxonomic approaches, namely, comparison of the relative distributions of homologous series of apparently universal occurrence, and

the establishment, by inference, of the presence or absence of enzyme systems responsible for the elaboration of specific highly complex compounds. The advantages of plant alkane distribution analysis in the light of the ubiquitous occurrence of these compounds and their position as end-products insulated from the internal metabolism of the plant as a means of "fingerprinting" individual species have been stressed by Eglinton and his colleagues,[1,10–12] whilst study of the presence or absence of compounds of a specific type as a chemotaxonomic principle has been well established in several fields, e.g. the work of Erdtman[13] on the terpenoids present in conifers and the work of Goutarel[14] on the steroidal alkaloids present in the Apocynaceae.

DISCUSSION

The total quantity of surface wax fell in the range of 0·3–0·6 per cent of the fresh leaf weight for all garden-grown *Cortaderias* with the highest percentage occurring in *C. toetoe* and the lowest in *C. atacamensis*. The three species indigenous to New Zealand had higher total surface wax content than the two South American species, as might have been anticipated from the visible differences in surface wax discussed earlier.

The composition of the waxes from the five garden-grown species were remarkably similar in overall composition with the percentage of total alkanes in the wax lying in the range 3·3–4·4 per cent, the percentage of total fatty acids lying in the range 45–48 per cent and the percentage of total *n*-alkanols lying in the range 20–23 per cent.

Alkane Distribution Patterns

The percentage of each alkane in the total alkane fraction is given in Table 1. Also included for comparative purposes in Table 1 are the corresponding data obtained from the original work[1] with Plimmerton *C. toetoe* collected in September 1959 and December 1961.

The results obtained with Plimmerton *C. toetoe* give clear indication of the occurrence of variation, both seasonal and between organs, in the percentage compositions of the alkane constituents of the surface wax. Thus there is a marked divergence in the compositions of the surface alkane fractions obtained from leaves collected at different dates (Sept. 1959, Dec. 1961, June 1965) and there is a variation in the compositions of the alkanes obtained from the leaves and from the roots and basal shoots in the June 1965 collection. Since the material collected in June and July would contain new leaves from the many autumn-emerged tillers, the seasonal variation may, in fact, reflect a variation with age. If so, it would offer an interesting parallel to the results obtained in preliminary studies on the presence of cyanogenetic glycosides in the five species of *Cortaderia* which have shown that these compounds, although present in young leaves, were not detectable in old leaves.[15]

The seasonal variation in leaf surface alkane composition of Plimmerton *C. toetoe* is also of interest in terms of experiments which have been reported on the total quantity of leaf surface wax present in the grass *Poa colensoi* Hook. f.[16] These have shown that the amount

[10] G. Eglinton, A. G. Gonzalez, R. J. Hamilton and R. A. Raphael, *Nature* **193**, 739 (1962).
[11] G. Eglinton, A. G. Gonzalez, R. J. Hamilton and R. A. Raphael, *Phytochem.* **1**, 89 (1962).
[12] G. Eglinton and R. J. Hamilton, In *Chemical Plant Taxonomy* (Edited by T. Swain), pp. 187–217. Academic Press, New York (1963).
[13] H. Erdtman, In *Perspectives in Organic Chemistry* (Edited by A. Todd), p. 473 *et seq.* Interscience, New York (1956).
[14] R. Goutarel, *Les Alcaloïdes Stéroidiques des Apocynacées*, Hermann, Paris (1964).
[15] H. E. Connor and E. P. White, Unpublished observations.
[16] G. T. Daly, *J. Exptl Botany* **15**, 160 (1964).

of wax present increases in inverse ratio to the quantity of rainfall and increases slightly with increase in temperature. It was also suggested[16] that wind might influence the total quantity of surface wax present. No determinations of the compositions of the wax were, however, attempted in the work with *Poa colensoi.*

TABLE 1. PERCENTAGE COMPOSITION OF SURFACE ALKANES OF *Cortaderia* SPECIES AS DETERMINED BY GAS–LIQUID CHROMATOGRAPHY ON 0·5% APIEZON "L" COLUMNS AT 240°C*†

Plant	Portion Extracted	*n*-Alkane Defined By Carbon Atom Content											
(A) New Zealand Species		C^{22}	C^{23}	C^{24}	C^{25}	C^{26}	C^{27}	C^{28}	C^{29}	C^{30}	C^{31}	C^{32}	C^{33}
C. toetoe, Sept. 1959, Plimmerton[1]	Dried ground leaves		+	+	4	2	9	5	60	5	12	2	3
C. toetoe, Dec. 1961, Plimmerton[1]	Leaves		+	+	7	6	14	9	50	4	10		
C. toetoe, June 1965, Plimmerton	Roots and basal shoots			6	+	6	6	8	51	3	9	6	
C. toetoe, June 1965, Plimmerton	Leaves	+	+	+	+	+	5	4	82	3	3		
C. toetoe, July 1965, Lincoln	Leaves	+	+	+	3	+	5	2	83	2	+		
C. fulvida, July 1965, Lincoln	Leaves				+	+	3	9	80	2	4		
C. richardii, July 1965, Lincoln	Leaves	+	+	+	+	+	2	2	86	2	6		
(B) South American Species													
C. selloana, July 1965, Lincoln	Leaves				2	2	4	3	58	3	23	2	
C. atacamensis, July 1965, Lincoln	Leaves			+	2	3	9	4	29	+	39	13	
(C) *C. toetoe*, Mar. 1965, Raglan	Leaves	+	5	1	6	2	5	3	58	7	13		

* The content of an individual alkane is expressed as a mole percentage of the total hydrocarbon content from C_{22}–C_{33} inclusive. The mole percentage is taken as being equivalent to the area percentage, i.e. 100 $A_n/\Sigma_{22}^{33} A_n$, where A_n is the area of the peak corresponding to the hydrocarbon $C_nH_{(2n+2)}$, 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_{29} and C_{31}) and by log retention times.[17]

From the foregoing it is abundantly clear that considerable caution must be exercised in any attempted application of plant alkane analysis to taxonomy. Certainly it is apparent that a systematic investigation into the possible influence of season, climate, geographical distribution and the kind and age of organs on the composition of plant surface waxes is essential before the method can be accepted without qualification.

It is apparent from Table 1 that the July 1965 collections of all three garden-grown New Zealand species, *C. fulvida*, *C. richardii* and *C. toetoe*, have very similar leaf surface alkane distributions and this must raise considerable doubt as to the fulfilment of the original hopes[10,11] of employing plant alkane analysis as a rigorous species-diagnostic tool, even where variations in season, climate, geographical location and kind and age of organs are eliminated. Certainly comparison of the leaf alkane analysis of Plimmerton *C. toetoe* collected in June 1965 with the leaf alkane analyses of garden-grown *C. fulvida*, *C. richardii* and *C. toetoe* collected in July 1965 does not permit specific identification of this material.

The position with Raglan *C. toetoe* is complicated by probable seasonal variation as the plant material was collected in March 1965.

Nevertheless, the present work would appear to provide a distinction between the indigenous species of *Cortaderia* and the naturalized South American species, in that the former seem to have a lower percentage of the C_{31} component (as compared to the C_{29} component) in the leaf wax than the latter. Indeed, in South American *C. atacamensis* the C_{31} component is the major constituent rather than the C_{29} component which is the major constituent in the other four species.

It is noteworthy that none of the species of *Cortaderia* appeared to contain any branched alkanes since these compounds were not detectable by gas–liquid chromatographic analysis under the conditions employed. All the peaks obtained on the gas–liquid chromatographic traces fell on the one straight line when log retention time was plotted against carbon atom number.[17] Where isoalkanes are present, they give rise to a second straight line which is not coincident with the *n*-alkane line on a plot of log retention time against carbon atom number.[11,18,19]

In agreement with present concepts of biogenesis,[12] *n*-alkanes with an odd number of carbon atoms form the major alkane components.

Fatty Acids and n-*Alkanols*

The results of the analyses of the total fatty acid and total *n*-alkanol content of the surface waxes, as determined by gas–liquid chromatography of the derived methyl esters and the derived acetates, respectively, on 10% PEGA columns at 175° are shown in Tables 2 and 3.

TABLE 2. PERCENTAGE DISTRIBUTION OF FATTY ACIDS IN SURFACE WAX COMPONENTS OF *Cortaderia* SPECIES AS DETERMINED BY GAS–LIQUID CHROMATOGRAPHY OF THE DERIVED METHYL ESTERS ON 10% PEGA COLUMNS AT 175°C*

Plant	Portion Extracted	Lauric Acid	Myristic Acid	Palmitic Acid	Stearic Acid	Oleic Acid
(A) New Zealand Species						
C. toetoe, June 1965, Plimmerton	Roots and basal shoots	26	20	32	15	8
C. toetoe, June 1965, Plimmerton	Leaves	22	19	39	14	6
C. toetoe, July 1965, Lincoln	Leaves	24	29	39	8	
C. fulvida, July 1965, Lincoln	Leaves	28	26	32	13	
C. richardii, July 1965, Lincoln	Leaves	26	30	39	14	
(B) South American Species						
C. selloana, July 1965, Lincoln	Leaves	23	28	40	9	
C. atacamensis, July 1965, Lincoln	Leaves	24	26	37	13	

* The content of an individual acid is expressed as a percentage of the total acid content. Percentages were obtained by gravimetric integration of the areas of the recording paper enclosed under the peaks on the trace of the derived methyl esters.

[17] A. T. JAMES, In *Methods of Biochemical Analysis* (Edited by D. GLICK), Vol. 8, pp. 1–59. Interscience, New York (1960).
[18] D. T. DOWNING, Z. H. KRANZ and K. E. MURRAY, *Australian J. Chem.* **13**, 80 (1960).
[19] E. J. LEVY, R. R. DOYLE, R. A. BROWN and F. W. MELPOLDER, *Analyt. Chem.* **33**, 698 (1961).

TABLE 3. PERCENTAGE DISTRIBUTION OF *n*-ALKANOLS IN SURFACE WAX COMPONENTS OF *Cortaderia* SPECIES AS DETERMINED BY GAS–LIQUID CHROMATOGRAPHY OF THE DERIVED ACETATES ON 10% PEGA COLUMNS AT 175°C*

Plant	Portion Extracted	Hexan-1-ol	Octan-1-ol	Decan-1-ol	Dodecan-1-ol	Tetradecan-1-ol
(A) New Zealand Species						
C. toetoe, June 1965, Plimmerton	Roots and basal shoots	20	28	39	12	9
C. toetoe, June 1965, Plimmerton	Leaves	22	29	41	6	2
C. toetoe, July 1965, Lincoln	Leaves	26	20	38	14	
C. fulvida, July 1965, Lincoln	Leaves	24	19	40	17	
C. richardii, July 1965, Lincoln	Leaves	23	26	38	13	
(B) South American Species						
C. selloana, July 1965, Lincoln	Leaves	18	29	42	19	
C. atacamensis, July 1965, Lincoln	Leaves	26	24	38	20	

* The content of an individual alcohol is expressed as a percentage of the total alcohol content. Percentages were obtained by gravimetric integration of the areas of the recording paper enclosed under the peaks on the trace of the derived acetates.

These results would, however, seem of little value in providing a chemotaxonomic distinction among the five species of *Cortaderia*. In no case did the total number of acids, nor the total number of alcohols, exceed five, whilst the same four acids (lauric, myristic, palmitic and stearic acids, i.e. the usual acids of the glyceride pool) and the same four alcohols (*n*-hexanol, *n*-octanol, *n*-decanol and *n*-dodecanol) were present in all five species in comparable relative amounts. Moreover, palmitic acid was the preponderant acid and *n*-decanol was the preponderant alcohol in all cases.

The detection of the unsaturated acid, oleic acid, together with tetradecanol in Plimmerton *C. toetoe* (leaves; shoots and roots) and its apparent absence from other species (especially garden-grown *C. toetoe* from Lincoln) may reflect environmental differences, or may result from sampling error since far greater quantities of total extract were available from the Plimmerton material than were available from the others. Seasonal variation would seem less probable here since the 1965 Plimmerton material and the five garden-grown species were all collected at about the same time of year.

A noteworthy feature of Table 3 is the short chain lengths of the alcohols (C_6–C_{14}). The chain lengths of the acids in Table 2 (C_{12}–C_{18}) in relation to the chain lengths of the *n*-alkanes in Table 1 (C_{23}–C_{33}) are as to be expected in terms of current biogenetic theory in which it is considered[12] that one route at least leading to the formation of *n*-alkanes involves the coupling of two molecules of fatty acid before decarboxylation and reduction to the paraffin occurs—much as in the established biogenesis of corynomycolic acid.[20]

The results of the acid and alcohol analyses show an apparent restriction of these compounds to those with an even number of carbon atoms, which would again be in accord with current biogenetic theory.[12]

Triterpene Methyl Ethers

Infra-red analyses of the total light petroleum extractives from the surface wax of each *Cortaderia* revealed the presence of absorption at 1104 cm^{-1}. characteristic of the ether

[20] M. GASTAMBIDE-ODIER and E. LEDERER, *Nature* **184**, 1563 (1959).

function, only in *C. fulvida*, *C. richardii*, garden-grown *C. toetoe* and Plimmerton *C. toetoe* (leaves; shoots and roots) and its absence in the two South American species and Raglan *C. toetoe*. However, in order to check the possibility that trace amounts of triterpene ethers, insufficient to reveal themselves in the infrared spectra of the total light petrol extracts, could still be present in the South American species or the Raglan material, the light petrol extracts from these plants were worked up as if they did contain triterpene ethers. Since no ethers could be detected at the appropriate stage in the alumina column chromatography it can be concluded that the South American species and the Raglan *C. toetoe* contain no appreciable quantities of triterpene methyl ethers.

The initial triterpene methyl ether fractions eluting in the alumina column chromatography of the extractives of *C. fulvida*, *C. richardii*, garden-grown *C. toetoe* and Plimmerton *C. toetoe* were in each case identical (i.r. spectra, absence of mixed m.p. depression) with authentic arundoin. However, the later fractions from both garden-grown and Plimmerton *C. toetoe* showed m.p.'s lower than that of pure arundoin, and in accord with the earlier work[3] were considered to be mixtures.

Application of gas–liquid chromatography using 0·5% Apiezon L as stationary phase showed that only one peak, corresponding in retention time to arundoin, was present in the total triterpene methyl ether fractions from both *C. fulvida* and *C. richardii* but that the total triterpene methyl ether fraction from *C. toetoe* gave rise to three peaks on the trace.

The five garden-grown species of *Cortaderia* can thus be divided into three classes on the basis of the triterpene methyl ether analysis:

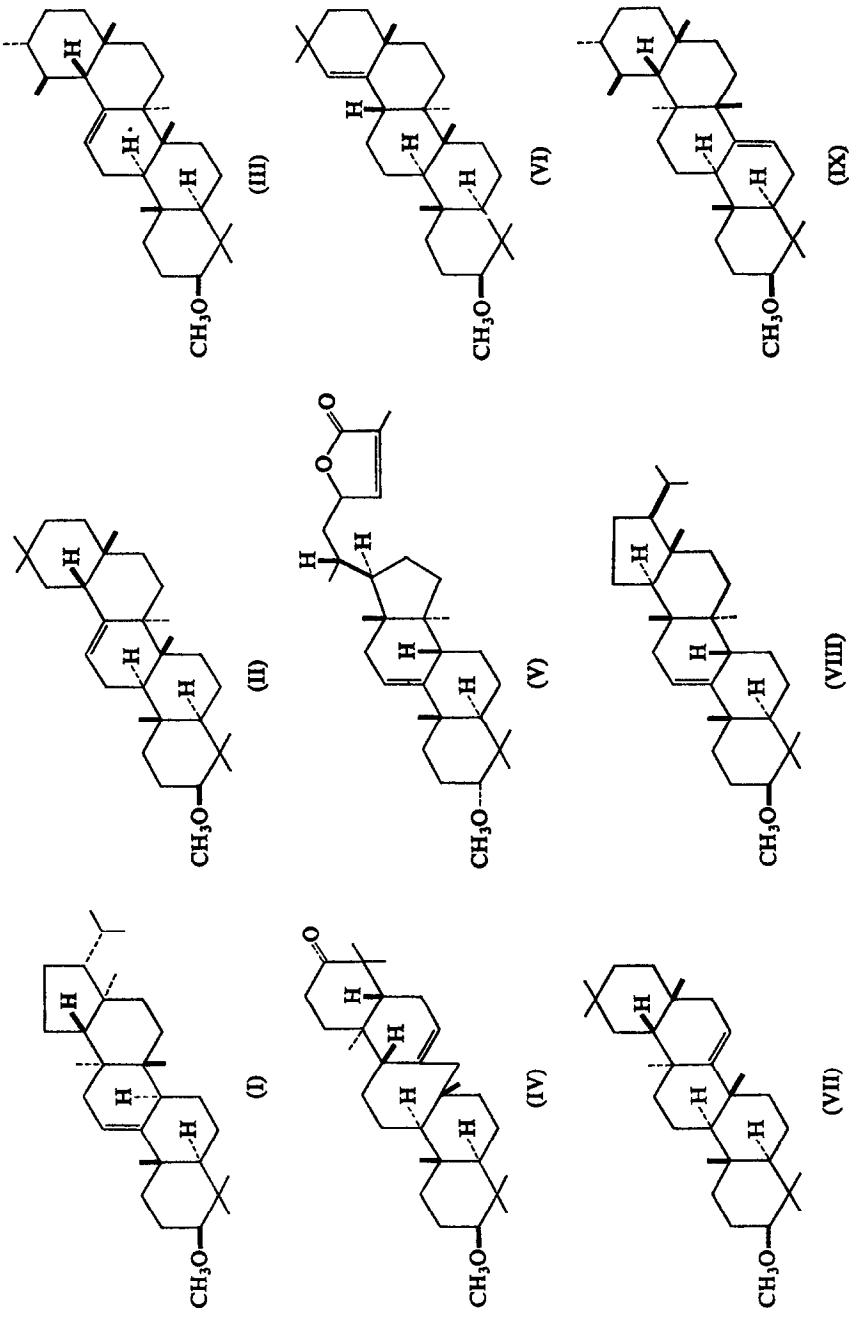
(i) the two South American species which contain no triterpene methyl ethers;
(ii) *C. fulvida* and *C. richardii* which each contain arundoin (I) as the sole triterpene methyl ether; and
(iii) *C. toetoe* which contains arundoin and at least two other triterpene methyl ethers.

Application of preparative gas–liquid chromatography and mass spectrometry, as described in our subsequent paper,[4] showed that only three triterpene methyl ethers were indeed present in both garden-grown and Plimmerton *C. toetoe* and that these compounds were arundoin (I), β-amyrin methyl ether (II) and α-amyrin methyl ether (III).

CONCLUSIONS

Studies on the constituents of the leaf surface waxes of five species of *Cortaderia*, obtained by cold light petrol extraction of the unmacerated fresh leaves, have indicated a certain degree of chemical differentiation. Although the fatty acid and *n*-alkanol analyses show no separation at the specific level, and the alkane analyses only effectively distinguished *C. atacemensis* from the other four species, the triterpene methyl ether analyses do indicate some clear distinctions. In *C. toetoe* arundoin and the methyl ethers of α-amyrin and β-amyrin are present; the two other New Zealand species, *C. fulvida* and *C. richardii*, contain arundoin only; and in the two South American species, *C. atacamensis* and *C. selloana* no triterpene methyl ethers are present. Since the alkane distribution patterns would appear to differentiate *C. selloana* from *C. atacamensis*, this work has failed only in not finding a chemical distinction between *C. fulvida* and *C. richardii*. Indeed, these latter two taxa were, in the past, considered by some authors as varieties of one species. This view is rarely accepted today, although Connert[21] recognized only one indigenous species. Artificial F_1 hybrids

[21] H. J. Connert, *Die Sytematik und Anatomie der Arundineae*, Cramer, Weinheim (1961).


(I)
(II)
(III)
(IV)
(V)
(VI)
(VII)
(VIII)
(IX)

C. richardii ♀ × *C. fulvida* ♂ appear to be fertile while the recessive gene for male sterility recognized in *C. richardii*[22] functions efficiently in the F_1 hybrid genetic complement. Genetic differentiation in these two decaploid species is apparently not highly evolved.

This study has also served to show that, chemically, the Plimmerton material is identical with garden-grown *C. toetoe*, but the position with respect to the Raglan material, also identified as *C. toetoe*, is unsatisfactory, especially in the light of the absence of triterpene methyl ethers. *C. toetoe* from the Waikato Province differs in some small respects from typical *C. toetoe* from Wellington Province, but not in such a way as to suggest that it deserves even infraspecific recognition. Although three species of *Cortaderia* occur in the Raglan district—*C. fulvida*, *C. toetoe* and *C. selloana*—hybridization between *C. toetoe* and *C. selloana* would seem highly improbable for the various cytological, ecological and phenological reasons outlined by Connor,[9] and could not be readily invoked to account for the absence of triterpene methyl ethers in this collection.

Raglan *C. toetoe* could perhaps be a mutant since production of the triterpene methyl ethers in indigenous New Zealand *Cortaderia* seems to be under genetic control and not generally influenced by the environment as evidenced by the fact that *C. toetoe* from its typical swampy sites at Plimmerton produced the same three ethers as transplants growing for some years in an open garden on a well-drained soil under much lower rainfall. The genetic system controlling the inheritance of the enzyme systems responsible for triterpene methyl ether synthesis is unknown but could be studied from suitable experiments with interspecific hybrids particularly between *C. richardii* and *C. toetoe*.

A possible parallel to the occurrence of triterpene methyl ethers in the garden-grown and the Plimmerton *C. toetoe* and the absence of these compounds from Raglan *C. toetoe* is provided by the recent isolation[23] of triterpene methyl ethers from Cuban sugar cane (*Saccharum officinarum* L.) and the apparent absence of such compounds in sugar cane grown in other geographical locations.[24, 25] However, since the detection of trace amounts of triterpene methyl ethers in plant material has only become possible in recent years with the development of modern methods of analysis—especially i.r. spectroscopy and refined chromatographic techniques—much of the work with sugar cane wax would not necessarily have pointed to the presence of these compounds. Since triterpene methyl ethers have physical properties very akin to those of the paraffins, it is possible that they were rejected with the alkane fraction during preliminary chemical work-up of the sugar cane wax, and so re-investigation with modern techniques is essential before any final conclusion can be made as to the true absence of triterpene methyl ethers from a given sample of sugar cane wax. Nevertheless, an analysis of the hydrocarbons present in Australian sugar cane wax by means of gas–liquid chromatography[25] failed to reveal the presence of triterpene methyl ethers in a situation apparently comparable to that giving the first intimation of the presence of triterpene methyl ethers in *Cortaderia toetoe*.[1]

[22] H. E. Connor, *New Zealand J. Botany* **3**, 233 (1965).

[23] T. A. Bryce, M. Martin-Smith, G. Osske, K. Schreiber and G. Subramanian, *Tetrahedron*, In press.

[24] *Inter alia* R. T. Balch, *Wax and Fatty By-Products From Sugar Cane*, Tech. Rep. Ser. No. 3 (Sugar Research Foundation Inc., New York) (1947); D. E. Whyte and B. Hengeveld, *J. Am. Oil Chem. Soc.* **27**, 57 (1950); N. Wiedenhof, *J. Am. Oil Chem. Soc.* **36**, 297 (1959); D. R. Kreger, *Rec. Trav. Bot. Neerl.* **41**, 606 (1948); D. H. S. Horn and M. Matic, *J. Sci. Food Agri.* **8**, 571 (1957); S. Bose and K. C. Gupta, *Proc. Ann. Conv. Sugar Technologists' Assoc., India* **29**, 70 (1961), in *Chem. Abstr.* **60**, 13423 (1964); J. A. Lamberton and A. H. Redcliffe, *Australian J. Chem.* **13**, 261 (1960); J. A. Lamberton and A. H. Redcliffe, *Australian J. Appl. Sci.* **11**, 473 (1960).

[25] Z. H. Kranz, J. A. Lamberton, K. E. Murray and A. H. Redcliffe, *Australian J. Chem.* **13**, 498 (1960).

One signal result from the present investigations is that *C. toetoe*, only recognized as a distinct species as recently as 1963,[6] is chemically markedly differentiated from *C. richardii* with which it was formerly treated under the misapplied name *Arundo conspicua* Forst. f.

The marked chemical difference between the three New Zealand species, which elaborate triterpene methyl ethers, and the two South American species which do not, is paralleled by equally marked morphological and physiological differences. Connert,[21] in the latest complete revision of the genus *Cortaderia*, placed *C. atacamensis* and *C. selloana* in Section Cortaderia while the New Zealand species are included, along with other South American species, in Section Bifida. An examination of the leaf surface waxes in the twenty untested South American species should be rewarding.

In the light of present knowledge it would appear that the occurrence of methyl ethers of pentacyclic triterpenes of the oleanane, ursane and hopane-isohopane types is confined to the Gramineae, although triterpene methyl ethers of other types occur in the Pinaceae. Thus the pentacyclic triterpene methyl ether IV, formally derived from serratenediol, has been isolated together with closely related compounds from the barks of certain pines[26] and the tetracyclic triterpene methyl ether, abieslactone, V, has been obtained from the bark and leaves of **Abies mariesii* Masters.[27] In addition to its occurrence in *C. toetoe* together with the methyl ether of β-amyrin (II) and the methyl ether of α-amyrin (III), in *C. fulvida* and in *C. richardii* as shown in the present work, arundoin (I) also occurs in **Imperata cylindrica*,[5,28] in **Zoysia japonica* Steud.[29] and in the wax of Cuban sugar cane (*Saccharum officinarum* L.).[23] Germanicol methyl ether (miliacin, VI)[30–32] occurs as a constituent of the grasses **Panicum miliaceum* L. and **Syntherisma sanguinalis* Dulac var. *ciliaris* Honda and taraxerol methyl ether (VII), known variously as crusgallin[30] and sawamilletin[33] although the former trivial name has precedence,[34] occurs in **Echinochloa crusgalli* Beauv. subsp. *edulis* Honda var. *typica* Honda and in Cuban sugar cane.[23] Isoarborinol methyl ether (cylindrin, VIII) has been obtained from *Imperata cylindrica*[5,28] and *Zoysia japonica*[29] and a triterpene methyl ether having gas–liquid chromatographic behaviour identical with that of bauerenol methyl ether (IX) is present in Cuban sugar cane wax.[23]

Thus these triterpene methyl ethers appear to be reasonably widespread in the grasses, occurring in the four tribes Cortaderiae (*Cortaderia fulvida, C. richardii, C. toetoe*), Zoysieae (*Zoysia japonica*), Paniceae (*Echinochloa crusgalli, Panicum miliaceum, Syntherisma sanguinalis*) and Andropogoneae (*Imperata cylindrica, Saccharum officinarum*).

The co-occurrence of arundoin (I), β-amyrin methyl ether (II) and α-amyrin methyl ether (III) in *Cortaderia toetoe*, of arundoin (I), taraxerol methyl ether (VII) and possibly bauerenol methyl ether (IX) in Cuban sugar cane wax and of arundoin (I) and cylindrin (VIII) in *Imperata*

* Species names with the authorities cited, are those used by the authors of the original papers; no attempt has been made to substitute modern equivalents.

[26] J. W. ROWE and C. L. BOWER, *Tetrahedron Letters*, No. 32, 2745 (1965).
[27] S. MATSUNAGA, J. OKADA and S. UYEO, *Chem. Commun.* 525 (1965).
[28] T. OHMOTO, K. NISHIMOTO, M. ITO and S. NATORI, *Chem. Pharm. Bull., Tokyo* **13**, 224 (1965).
[29] K. NISHIMOTO, M. ITO, S. NATORI and T. OHMOTO, *Chem. Pharm. Bull., Tokyo* **14**, 97 (1966).
[30] H. ITO, *J. Fac. Agr. Hokkaido Imp. Univ.* **37**, 1 (1934), in *Chem. Abstr.* **29**, 627 (1935).
[31] H. ITO, *J. Chem. Soc. Japan* **59**, 274 (1938), in *Chem. Abstr.* **32**, 9072 (1938).
[32] S. ABE, *Bull. Chem. Soc. Japan* **33**, 271 (1960), in *Chem. Abstr.* **55**, 3647 (1961); N. SUGIYAMA and S. ABE *J. Chem. Soc. Japan* **82**, 1051 (1961), in *Chem. Abstr.* **57**, 13810 (1962); S. ABE, *J. Chem. Soc. Japan* **82**, 1054, 1057 (1961), in *Chem. Abstr.* **57**, 13811 (1962).
[33] T. OBARA and S. ABE, *J. Chem. Soc. Japan* **80**, 677 (1959); S. ABE and T. OBARA, *J. Chem. Soc. Japan* **80**, 1487 (1959); S. ABE, *J. Chem. Soc. Japan* **80**, 1491 (1959), in *Chem. Abstr.* **55**, 3646 (1961).
[34] H. ITO, T. OBARA and S. ABE, *J. Chem. Soc. Japan* **86**, 540 (1965).

cylindrica is of interest in the light of modern theory of biogenesis of triterpenes,[35] since it would be in excellent agreement with the hypothesis that cyclizations of more than one conformational folding of squalene are occurring side by side in the same plant. Thus arundoin can be postulated to arise via chair, chair, chair, chair, boat cyclization of all *trans* squalene; the methyl ethers of α-amyrin, β-amyrin, taraxerol and bauerenol via chair, chair, chair, boat cyclization of all *trans* squalene; and cylindrin via chair, boat, chair, chair, boat cyclization of all *trans* squalene.[29, 36] The reason for the co-existence of more than one mode of cyclization of squalene in the one plant, which is probably of widespread occurrence since it would seem to occur whenever true plant sterols (chair, boat, chair, boat conformational sequence[35]) and triterpenes of the lupane, ursane or oleanane types (chair, chair, chair, boat conformational sequence[35]) exist in the same plant, is still unknown, as indeed is the precise role played by sterols and triterpenoids in plant economy. Examples of the co-occurrence of plant sterols and triterpenes are afforded by *Nerium odorum* (cardiac glycosides[37] and ursolic and oleanolic acids[38]), the soya bean (stigmasterol[39] and the soyasapogenols[40]), the pea (β-sitosterol and β-amyrin)[41], shea oil (parkeol[42] and butyrospermol[43]), and Cuban sugar cane (various sterols[44] and triterpene methyl ethers[23]).

MATERIAL AND METHODS

Plant Material

8·8 kg of fresh leaves (sheaths and blades) of the five species of *Cortaderia* were collected in July 1965 from plants growing in the uniform environment of the New Zealand D.S.I.R. Botany Division experimental gardens at Lincoln, Canterbury, New Zealand. *Cortaderia fulvida* was transplanted to Lincoln from the Rimutaka Range; *C. richardii* raised from seed from the Ashburton Gorge; *C. toetoe* transplanted from Palmerston North; *C. atacamensis* transplanted from Auckland; and *C. selloana* from Lincoln. Large quantities of roots and leaves of Plimmerton *C. toetoe* were collected in June 1965 from wild plants in a swamp there; large-scale extraction of arundoin (I) was made from this sample.

Specimens from all collections are deposited in the herbarium of the Botany Division, Department of Scientific and Industrial Research, Christchurch (CHR).

Isolation of Surface Waxes

Fresh leaves of each grass in varying quantities, as available, were cut into 25 cm lengths and immersed in re-distilled light petroleum (40–60°) for 16 hr at room temperature. The

[35] *Inter alia* L. RUZICKA, *Proc. Chem. Soc.* 341 (1959); T. G. HALSALL and R. T. APLIN, *Fortschritte der Chemie Organischer Naturstoffe* (Edited by L. ZECHMEISTER), Vol. 22, pp. 153–202 (1964); J. H. RICHARDS and J. B. HENDRICKSON, *The Biosynthesis of Steroids, Terpenes and Acetogenins*, pp. 257–288. Benjamin, New York (1964).

[36] O. KENNARD, L. RIVA DI SANSEVERINO, H. VORBRÜGGEN and C. DJERASSI, *Tetrahedron Letters*, No. 39, 3433 (1965).

[37] T. TAKEMOTO and K. KOMETANI, *J. Pharm. Soc. Japan* **74**, 1263 (1954). In *Chem. Abstr.* **49**, 4233 (1955); W. RITTEL, A. HUNGER and T. REICHSTEIN, *Helv. Chim. Acta* **36**, 434 (1953).

[38] M. ISHIDATE, Z. TAMURA and M. OKADA, *J. Pharm. Soc. Japan* **67**, 206 (1947). In *Chem. Abstr.* **45**, 9068 (1951).

[39] H. MATTHES and A. DAHLE, *Arch. Pharm.* **249**, 436 (1911).

[40] G. CAINELLI, J. J. BRITT, D. ARIGONI and O. JEGER, *Helv. Chim. Acta* **41**, 2053 (1958); H. M. SMITH, J. M. SMITH and F. S. SPRING, *Tetrahedron* **4**, 111 (1958).

[41] E. J. CAPSTACK, D. J. BAISTED, W. W. NEWSCHWANDER, G. BLONDIN, N. L. RASIN and W. R. NES, *Biochemistry* **1**, 1178 (1962).

[42] K. H. BAUER and H. MOLL, *Fette, Seifen, Anstrichmittel* 560 (1939); W. LAWRIE, F. S. SPRING and H. S. WATSON, *Chem. Ind.* 1458 (1956).

[43] M. C. DAWSON, T. G. HALSALL, E. R. H. JONES and P. A. ROBINS, *J. Chem. Soc.* 586 (1953).

[44] G. OSSKE and K. SCHREIBER, *Tetrahedron* **21**, 1559 (1965).

light petroleum extractives were then obtained as pale yellow waxes by decantation and removal of the solvent under reduced pressure on a rotary film evaporator. The yields of total wax from 8·8 kg of fresh leaves of each of the garden-grown species were *C. fulvida*, 8·5 g; *C. richardii*, 7·5 g; *C. toetoe*, 10·5 g; *C. atacamensis*, 6·0 g; *C. selloana*, 7·0 g.

Isolation and Gas–Liquid Chromatographic Analysis of The Alkane Fractions

In all cases the following procedure was followed.

Total light petroleum extractives (1·0 g) from each species were refluxed with an excess of 2,4-dinitrophenylhydrazine (1·0 g) and conc. HCl (0·5 ml) in ethanol (20 ml) for 2 hr to convert carbonyl compounds into 2,4-dinitrophenylhydrazones. The solvent was then removed under reduced pressure and the residue exhaustively extracted with re-distilled light petroleum (40–60°). After removal of the solvent, the light petroleum-soluble material was refluxed for 2 hr in aqueous ethanol (1:2, 20 ml) containing NaOH (1·0 g). The solution was taken to dryness under reduced pressure and the residue thoroughly extracted with light petroleum. The resulting solution was then chromatographed over basic alumina (Woelm grade I, 5 g) and the hydrocarbon fraction completely eluted with light petroleum of b.p. 40–60°. The residue obtained on removal of the solvent was subjected to i.r. analysis in KCl disc and if no absorption other than that due to alkanes was observed the sample was submitted directly to gas–liquid chromatographic analysis on a 0·5% Apiezon L column on the Pye Panchromatograph (*vide infra*).

Where i.r. absorption at 1104 cm^{-1} was present in the hydrocarbon fraction, indicating the presence of ethers, the alkane-containing fraction was treated with conc. H_2SO_4 (5 ml) at 140° for 4 hr before being taken up in re-distilled light petroleum and rechromatographed prior to gas–liquid chromatographic analysis.

After a satisfactory GLC trace had been obtained, authentic *n*-nonacosane was added to the natural alkane mixture and a second GLC trace obtained with the new mixture, with identification of the peak which was intensified with respect to the first trace. Repetition with addition of authentic *n*-untriacontane in place of the *n*-nonacosane permitted identification of the peak in the natural mixture due to the C_{31} *n*-alkane. Once these two peaks had been identified in this way, the remaining *n*-alkanes of the natural mixture were identified by taking advantage of the fact that a plot of log retention time against carbon atom number for an homologous series is a straight line.[17] Integration of the areas under each peak on the gas–liquid chromatographic trace of the natural mixture then permitted determination of the mole percentages of the individual alkanes.

The weights of the alkane fractions from 1 g of total light petroleum extractives were *C. fulvida*, 39 mg; *C. richardii*, 42 mg; *C. toetoe*, 43 mg; *C. atacamensis*, 33 mg; *C. selloana*, 40 mg.

Analysis of Fatty Acids and n-*Alkanols in Surface Wax Components*

Total light petroleum extractives (1 g) from each species were refluxed for 2 hr in aqueous ethanol (1:2, 20 ml) containing NaOH (3 g). The solution was taken to dryness and thoroughly extracted with dry ether. The combined ethereal solutions were then taken to dryness and the residue refluxed in acetic anhydride (5 ml) for 4 hr to convert the constituent alcohols into the corresponding acetates. The reaction mixture was allowed to cool to room temperature, water (20 ml) added and the solution left to stand for 24 hr to hydrolyse the excess of acetic anhydride. The mixture was then carefully neutralized with sodium bicarbonate solution and extracted with ether. Removal of solvent from the resulting ethereal solution afforded the mixed acetates ready for GLC analysis.

Yields of mixed acetates were *C. fulvida*, 291 mg; *C. richardii*, 289 mg; *C. toetoe*, 307 mg; *C. atacamensis*, 282 mg; *C. selloana*, 280 mg.

The ether-insoluble residue resulting from the aqueous ethanolic saponification of the light petroleum extractives was taken up in water (30 ml), the solution acidified to liberate the free carboxylic acids from their sodium salts, and extracted with ether to permit isolation of the acids. After removal of solvent from the ethereal solution the residual material was dissolved in methanol and the resulting solution treated with an excess of an ethereal solution of diazomethane. Removal of solvents under reduced pressure then afforded the corresponding methyl esters ready for GLC analysis.

Yields of mixed methyl esters were *C. fulvida*, 501 mg; *C. richardii*, 475 mg; *C. toetoe*, 502 mg; *C. atacamensis*, 481 mg; *C. selloana*, 482 mg.

The GLC analyses of both the alcohol acetates and the methyl esters of the acids were conducted on 10% PEGA columns at 175° (*vide infra*). Authentic *n*-hexyl acetate, *n*-octyl acetate and *n*-decyl acetate were employed to aid identification of the unknown acetates, through intensification experiments analogous to those described for the GLC alkane analyses described above. Similarly authentic methyl laurate, methyl palmitate, methyl stearate and methyl oleate were employed in analogous fashion to aid identification of the unknown methyl esters.

For the alcohol acetates, all the peaks fell on the one straight line when log retention time was plotted against carbon atom number, whilst the only peak not falling on the straight line for the analogous plot for the methyl esters was that identified as methyl oleate by addition of authentic material.

Integration of the areas under the peaks (using a gravimetric procedure) afforded the percentage of each component present.

Isolation of Triterpene Methyl Ethers

Total light petroleum extractives (2·0 g) from each species, whether or not they exhibited characteristic ether absorption at 1104 cm^{-1} in the i.r., were dissolved in light petroleum of b.p. 40–60° and chromatographed over neutral alumina (Woelm, grade I, 50 g), using further light petroleum of b.p. 40–60° to develop the column. The initial eluants (200 ml) in all cases contained alkanes but with *C. fulvida*, *C. richardii*, *C. toetoe* and Plimmerton *C. toetoe* further elution with light petroleum (500 ml) afforded a crystalline triterpene methyl ether fraction.

The triterpene methyl ether fraction (34 mg) from *C. fulvida* and the triterpene methyl ether fraction (38 mg) from *C. richardii* appeared homogeneous on gas–liquid chromatography using 0·5% Apiezon L at 240° and on crystallization from ethyl acetate had m.p. 235–237°, undepressed on admixture with authentic arundoin. Infrared spectra in KCl disc of all specimens were identical.

The triterpene methyl ether fraction (44 mg) from garden-grown *C. toetoe* and the triterpene methyl ether fraction (42 mg) from Plimmerton *C. toetoe* on gas–liquid chromatography 0·5% Apiezon L at 240° were each resolved into three components. Rechromatography over neutral alumina (Woelm, grade I, 50 g) using light petroleum of b.p. 40–60° afforded pure arundoin (12 mg) of m.p. 235–237°, identical with authentic material, as the first fractions to be eluted.

Gas–Liquid Chromatography

The instrument employed for all the analytical gas–liquid chromatographic studies was a standard Pye Panchromatograph, giving preheating of the argon carrier gas and fitted with

standard glass tubes, containing the column packing, of 150 × 0·45 cm. The detector was the standard Lovelock argon ionization type, fitted with a 90Sr source and the current from the detector was fed into a Honeywell Brown (Newhouse, Lanarkshire, Scotland) pen recorder with sensitivity 0–10 mV.

Direct injections (0·2–0·3μl. of a chloroform solution of the compounds under investigation) were made on to the column through a silicone-rubber "blind hole" stopper with a 1 μl. syringe (Hamilton Co. Inc., Whittier, Calif., U.S.A.). Standard conditions were as follows: column temperature, 240 ± 1° for Apiezon L columns, 175° for polyethyleneglycol adipate (PEGA) columns; detector temperature 248 ± 1° for Apiezon L columns, 190° for polyethyleneglycol adipate columns; argon flow rate 60 ml/min at outlet (inlet pressure 10–12 lb/in^2); nominal detector voltage, 1000 V; sensitivity setting 1×10^{-8} A.

Preparation of Columns

Column packings for the Pye Panchromatograph were prepared on the silane-treated support, Gas-Chrom Z (Applied Science Laboratories Inc., State College, Pennsylvania, U.S.A.) of 100–120 mesh. The coating with stationary phase was achieved by weighing out the required quantity of the desired stationary phase, viz. Apiezon L grease (Edwards High Vacuum Ltd., Manor Royal, Crawley, Sussex, U.K.) or polyethyleneglycol adipate (Pye Instruments Ltd., Cambridge, U.K.), dissolving in AnalaR chloroform and adding the calculated amount of support to the solution so obtained. The chloroform was then removed by distillation *in vacuo* at 100° with the minimum of agitation and the coated supporting phase further dried for 1 hr *in vacuo* at 100°C. Column packings so prepared contained 0·5% (w/v) Apiezon L and 10% (w/v) PEGA.

The glass tubes were then filled with the required column packing with repeated gentle tapping. Before any freshly packed column was used for chromatography it was stabilized by heating at a temperature 10° above that to be used experimentally (i.e. 250° for Apiezon L columns and 185° for PEGA columns) for 24 hr in a slow stream of argon.

Determination of Retention Data

Measurements of retention times were made between the first displacement of the recorder pen after the injection and the point corresponding to the peak of the response to the compound concerned. The recorded response to the injection was observed 20–25 sec after the moment of injection and coincided with the return of the outlet flow rate from an elevated level (due to the pressure wave from the evaporation of chloroform) to 60 ml/min. 5α-Cholestane was included in solutions of triterpene methyl ethers as an internal standard and the retention times of these compounds were expressed as ratios relative to 5α-cholestane.

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