Phytochemistry, 1972, Vol. 11, pp. 1989 to 2001. Pergamon Press. Printed in England.

# THE CONSTITUENT ACIDS OF GYMNOSPERM CUTINS*

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(*Received* 8 *July* 1971, *in revised form* 8 *December* 1971)

**Abstract**—The constituent cutin acids of selected species of gymnosperms, a lycopod and a fern have been determined by GLC–MS of the methyl ester trimethylsilyl ethers. A large number of novel cutin acids has been found. Of particular significance is the occurrence of 9,16-dihydroxyhexadecanoic acid in certain species. The results suggest that cutin acid composition may be a useful taxonomic and phylogenetic criterion.

## INTRODUCTION

CUTIN is a major polymeric constituent of the non-cellular membrane, the cuticle, covering the aerial parts of plants.[1] It was shown by Matic[2] to be composed almost entirely of inter-esterified aliphatic hydroxy acids. The constituent acids of various cutins were subsequently studied by TLC[3–5] and GLC.[6,7] We have demonstrated the use of GLC–MS in the analysis of the methyl ester trimethylsilyl (TMSi) ethers of the cutin acids of apple fruit,[8] a 5000-year-old sediment[9] and a 30-million-year-old coal.[10] Hitherto, no work on the cutin acids of plants other than angiosperms has been published.

## RESULTS

Each cutin was prepared by the method of Baker *et al.*;[11] viz, removal of surface waxes with methanol, loosening of the cutin with oxalic acid–ammonium oxalate solution, removal of cellulose with zinc chloride–HCl solution and final washing in distilled water, MeOH and $CHCl_3$–MeOH. The prepared cutins were then hydrolysed with 5% KOH in MeOH and the reaction mixture worked up as follows: evaporation of MeOH, acidification with aqueous HCl extraction of the ether-soluble acids and methylation with diazomethane. The ester fraction was then silylated with bis(trimethylsilyl)acetamide and the component compounds determined by GLC and GLC–MS. A typical gas chromatogram of cutin acids, methyl esters, TMSi ethers (from *Gnetum gnemon*) is shown in Fig. 1. The identification of each component was based on its retention index, by either direct comparison with authentic compounds or by calculation from known compounds and/or its mass spectrum determined by GLC–MS. An example of a typical GLC–MS trace is given in Fig. 2. In no case was an

* Part IV in the series "Gas Chromatographic–Mass Spectrometric Studies of Long Chain Hydroxy Acids". For Part III see G. EGLINTON, D. H. HUNNEMAN and A. MCCORMICK, *Org. Mass Spec.* **1**, 593 (1968).

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[1] J. T. MARTIN and B. E. JUNIPER, *The Cuticles of Plants*, Edward Arnold, London (1970).
[2] M. MATIC, *Biochem. J.* **63**, 168 (1956).
[3] E. A. BAKER and J. T. MARTIN, *Nature, Lond.* **199**, 1268 (1963).
[4] E. A. BAKER and J. T. MARTIN, *Ann. Appl. Biol.* **60**, 313 (1967).
[5] C. H. BRIESKORN and J. BÖSS, *Fette Seifen, Anstrichmittel* **66**, 925 (1964).
[6] C. H. BRIESKORN and H. REINATZ, *Z. Lebensmittelunters. und Forsch.* **136**, 55 (1967).
[7] E. A. BAKER and P. J. HOLLOWAY, *Phytochem.* **9**, 1557 (1970).
[8] G. EGLINTON and D. H. HUNNEMAN, *Phytochem.* **7**, 313 (1968).
[9] G. EGLINTON, D. H. HUNNEMAN and K. DOURAGHI-ZADEH, *Tetrahedron* **24**, 5929 (1968).
[10] D. H. HUNNEMAN and G. EGLINTON, in *Advances in Organic Geochemistry 1968*, p. 157, Pergamon Press, Oxford (1969).
[11] E. A. BAKER, R. F. BATT and J. T. MARTIN, *Ann. Appl. Biol.* **53**, 59 (1964).

identification made without confirmation by a mass spectrum since a number of compounds were found to have identical retention indices. In particular, it was impossible to distinguish by GLC alone those compounds which differed only in having the hydroxyl group on the C-9 or C-10 position.

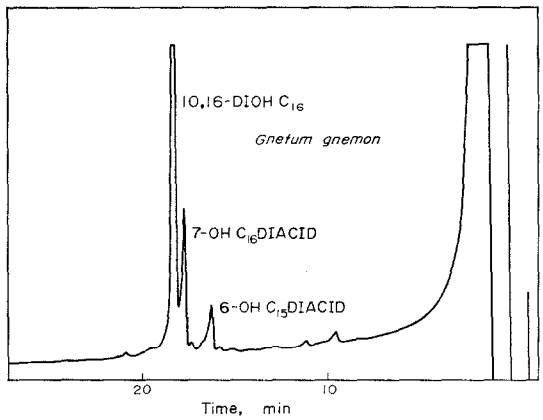



FIG. 1.

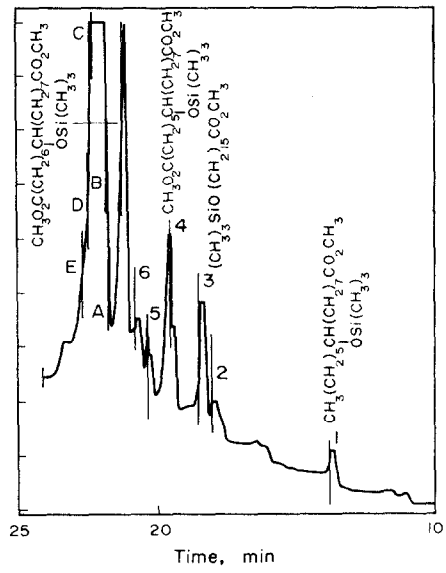



FIG. 2.

FIG. 1. GLC TRACE OF HYDROXY ACIDS (METHYL ESTER TMSi ETHERS) FROM THE HYDROLYSIS OF THE CUTIN OF *Gnetum gnemon*.

Conditions: column; 10 × 1/16 in o.d. stainless steel packed with 1% SE-30 on Gas Chrom Q; programmed 120–280° at 5°/min.

FIG. 2. GLC–MS TRACE (TOTAL ION CURRENT) OF HYDROXY ACIDS (METHYL ESTERS, TMSi ETHERS) FROM *Araucaria imbricata* CUTIN HYDROLYSIS.

The vertical lines through peaks indicate where a mass spectrum has been taken. The largest peak is mixture of two compounds and the partial spectra taken at A–E are shown in Fig. 15. Conditions: 10 ft × 1/8 in glass column packed with 1% SE-30 on Gas Chrom Q programmed 100–250° at 4°/min.

Some of the cutins were more difficult to purify than most angiosperm cutins and in the case of the conifers it was easier to scrape off a lot of adhering material rather than to depend on chemical extraction. Apparently conifer cuticles may differ somewhat in structure from the better known anigiosperm cuticles.

The distribution of the cutin acids found in each plant is given in Table 1 in tabular form as percentages of the most abundant component. The relative amounts of each cutin acid were determined by measuring the area under the GLC peak of the methyl ester TMSi ether. Areas were determined by multiplying peak heights by width at half peak height. This method, of course, does not take into account varying response factors but was considered adequate for the present purposes. Conversion of the hydroxy acids to the methyl ester TMSi ethers appears to be complete and thus derivatization was complete and non-discriminatory. No TMSi esters were observed, indicating completeness of esterification with diazomethane and no free hydroxy compounds were observed indicating completeness of silylation.

[12] R. F. SCAGET *et al.*, *An Evolutionary Survey of the Plant Kingdom*, Blackie, London (1965).

TABLE 1. CUTIN ACID COMPOSITION OF THE PLANTS STUDIED

The following conventions and abbreviations have been used in indicating the structure of the acids from leaf cutin hydrolysates: (1) unless otherwise indicated all compounds are straight chain acids; (2) chain length is indicated thus $C_{16}$; (3) position and number of hydroxyls is given before the chain length; (4) the number of double bonds is indicated thus $\Delta_n$. For example, 9,10, 18-triOH $\Delta_1 C_{18}$ is 9,10,18-trihydroxyoctadecenoic acid with the position of the double bond undefined.

| Cutin acid | Relative abundance (most abundant acid set at 100) | Cutin acid | Relative abundance (most abundant acid set at 100) |
|---|---|---|---|
| Lycopodophyta | | | |
| *Selaginella caniculata* | | | |
| $C_{16}$ | 14 | Unident. | 21 |
| 16-OH $C_{16}$ | 100 | | |
| Pterophyta | | | |
| *Anemia phyllitides* | | | |
| $C_{16}$ | 16 | 18-OH $C_{18}$ | 2 |
| $\Delta_1 + \Delta_2$ $C_{18}$ | 10 | $C_{22}$ | 18 |
| $C_{18}$ | 5 | $C_{24}$ | 6 |
| Unident. | 5 | 22-OH $C_{22}$ | 9 |
| 16-OH $C_{16}$ | 100 | $C_{28}$ | 5 |
| $C_{20}$ | 4 | $C_{28}$ alcohol | 7 |
| Unident. | 4 | $C_{30}$ alcohol | 7 |
| 9, 16-diOH $C_{16}$ | 3 | $C_{32}$ alcohol | 5 |
| 10,16-diOH $C_{16}$ | 5 | | |
| Cycadophyta | | | |
| *Encephalartos altensteinii* | | | |
| $C_{16}$ | 3 | 10,16-diOH $C_{16}$ | 100 |
| 14-OH $C_{14}$ | 13 | $C_{22}$ | 12 |
| 16-OH $C_{16}$ | 17 | Unident. | 3 |
| 7-OH $C_{15}$ diacid | 5 | Unident. | 17 |
| 6-OH $C_{15}$ diacid | 7 | $C_{24}$ | 3 |
| 8-OH $C_{16}$ diacid | 8 | 9,10,18-triOH $C_{18}$ (*erythro*) | 17 |
| 7-OH $C_{16}$ diacid | 2 | 9,10,18-triOH $C_{18}$ (*threo*) | 17 |
| 18-OH $C_{18}$ | 10 | | |
| 9,16-diOH $C_{16}$ | 75 | | |
| Ginkophyta | | | |
| *Ginkgo biloba* | | | |
| $C_{16}$ | 21 | Unident. | 5 |
| 16-OH $\Delta_1$ $C_{16}$ | 12 | 8-OH $C_{16}$ diacid | 2 |
| 16-OH $C_{16}$ | 34 | 7-OH $C_{16}$ diacid | 6 |
| 7-OH $C_{15}$ diacid | 3 | 9,16-diOH $C_{16}$ | 32 |
| 6-OH $C_{15}$ diacid | 6 | 10,16-diOH $C_{16}$ | 100 |
| Coniferophyta | | | |
| *Araucaria imbricata* | | | |
| 9-OH $C_{15}$ | 5 | 8-OH $C_{16}$ diacid | 10 |
| 16-OH $C_{16}$ | 8 | 9,16-diOH $C_{16}$ | 40 |
| 7-OH $C_{15}$ diacid | 10 | 10,16-diOH $C_{16}$ | 100 |
| 9,15-diOH $C_{15}$ | 3 | | |
| *Pinus sylvestris* | | | |
| 12-OH $C_{12}$ | 19 | 9,16-diOH $C_{16}$ | 100 |
| $C_{16}$ | 22 | 9-OH $C_{18}$ diacid | 1 |
| 14-OH $C_{14}$ | 9 | 9,18-diOH $C_{18}$ | 2 |
| 16-OH $C_{16}$ | 22 | 9,10,18-triOH $C_{18}$ (*erythro*) | 14 |
| 7-OH $C_{15}$ diacid | 3 | 9,10,18-triOH $C_{18}$ (*threo*) | 9 |
| 8-OH $C_{16}$ diacid | 3 | | |

| Cutin acid | Relative abundance (most abundant acid set at 100) | Cutin acid | Relative abundance (most abundant acid set at 100) |
|---|---|---|---|
| Gnetophyta | | | |
| *Gnetum gnemon* | | | |
| 6-OH $C_{15}$ diacid | 10 | 10,16-diOH $C_{16}$ | 100 |
| 7-OH $C_{16}$ diacid | 27 | | |
| *Welwitschia mirabilis* | | | |
| $C_{16}$ | 5 | 6-OH $C_{15}$ diacid | 13 |
| 10-OH $\Delta_1$ $C_{14}$ | 53 | Unident. | 15 |
| Unident. | 7 | 7-OH $C_{16}$ diacid | 34 |
| 16-OH $C_{16}$ | 2 | 10,16-diOH $C_{16}$ | 100 |
| Antophyta | | | |
| *Sapindus saponaria* | | | |
| 7-OH $C_{15}$ diacid | 2 | 7-OH $C_{16}$ diacid | 15 |
| 6-OH $C_{15}$ diacid | 10 | 9,16-diOH $C_{16}$ | 25 |
| 8-OH $C_{16}$ diacid | 5 | 10,16-diOH $C_{16}$ | 100 |
| *Ribes grossularia* (fruit) | | | |
| 9,16-diOH $C_{16}$ | 20 | 10,16-diOH $C_{16}$ | 100 |

## DISCUSSION

Probably the single most outstanding feature of the results reported here is the ability of the GLC–MS technique to determine the structure of a number of previously unreported cutin acids with ease and rapidity. With the use of the TLC[3–5] or GLC[6,7] alone, the existence of 9,16-dihydroxyhexadecanoic acid which occurs to varying amounts along with the well-known[2] 10,16-dihydroxyhexadecanoic acid remained unsuspected.

While insufficient data have been accumulated to make a detailed discussion of cutin acid composition versus taxonomy worthwhile, a few tentative remarks can be made. On the basis of the species studied so far, the following generalizations seem to be true: (1) 10,16-Dihydroxyhexadecanoic acid is the major acid in the cutins of all species of Gnetophyta and angiosperms. (2) 9-Hydroxylated compounds occur widely in gymnosperms other than the Gnetophyta and this tendency culminates in the complete absence of 10,16-dihydroxy-hexadecanoic acid and the dominance of 9,16-dihydroxyhexadecanoic acid in *Pinus sylvestris*. (3) In the ferns and lycopods, the dominating cutin acid appears to be 16-hydroxyhexadecanoic.

One can tentatively arrange a progression in cutin acid composition parallel to the evolution of the species studied, the higher the plant on the evolutionary scale the higher the degree of oxidation of the cutin acids. This principle is illustrated by the dominance of 16-hydroxyhexadecanoic acid in *Anemia phyllitides* and *Selaginella caniculata* and the large amount of trihydroxyacids in *Malus pumila*.[8] A study of a number of ferns and psilophytes could contribute significantly to the chemotaxonomic possibilities of cutin acid composition.

Because of their peculiar position on the borderline between gymnosperms and angiosperms, the Gnetophyta deserve some special mention. Unlike all the other gymnosperms

studied, the cutins of *Welwitschia mirabilis* and *Gnetum gnemon* lacked 9,16-dihydroxyhexadecanoic acid. The taxonomy of the Gnetophyta on the basis of their cutin acid composition is under further study.

In analysing the cutin acids, the entire ether soluble fraction from the acidified hydrolysate was obtained. Only carboxylic acids were found except with *Anemia phyllitides*, where the $C_{26}$, $C_{28}$ and $C_{30}$ long chain alcohols were also encountered. These compounds may be cutin components or incompletely extracted cuticular waxes. A more likely possibility is that there is no sharp delineation between cutin and the cuticular wax and that one layer extends into the other thus making the complete extraction of the wax extremely difficult, if not impossible.

TABLE 2. $C_{14}$–$C_{18}$ CARBOXYLIC ACIDS REPORTED OR POTENTIAL CUTIN COMPONENTS

The numbers given are the retention indices of the methyl ester TMSi ether on SE-30. Where the retention index is given in parenthesis this compound has been previously reported as a cutin component.

| Structural features | Chain length $C_{14}$ | $C_{15}$ | $C_{16}$ | $C_{17}$ | $C_{18}$ |
|---|---|---|---|---|---|
| *n*-Alkanoic | (1720) | — | (1920) | — | (2134) |
| $\Delta_1$ | — | — | — | — | (2110) |
| $\Delta_2$ | — | — | — | — | (2105) |
| 9-OH | — | 2054 | — | — | — |
| $\Delta_1$ | — | — | — | — | — |
| $\Delta_2$ | — | — | — | — | — |
| 10-OH | — | — | — | — | — |
| $\Delta_1$ | 1948 | — | — | — | — |
| $\Delta_2$ | — | — | — | — | — |
| $\omega$-OH | 2078 | — | (2278)[7] | — | — |
| $\Delta_1$ | — | — | 2254 | — | 2444 |
| $\Delta_2$ | — | — | — | — | 2424 |
| $\alpha, \omega$. diacid | — | — | (2260)[8] | — | 2460 |
| $\Delta_1$ | — | — | — | 2335 | 2435 |
| $\Delta_2$ | — | — | — | 2330 | 2430 |
| 9, $\omega$-diOH | — | 2373 | 2478 | — | 2680 |
| $\Delta_1$ | — | — | — | — | — |
| $\Delta_2$ | — | — | — | — | — |
| 10, $\omega$-diOH | — | — | (2478)[1,7] | — | (—)[7] |
| $\Delta_1$ | — | — | — | — | — |
| $\Delta_2$ | — | — | — | — | — |
| 9-OH diacid | — | 2340 | 2436 | — | 2645 |
| $\Delta_1$ | — | — | — | — | — |
| $\Delta_2$ | — | — | — | — | — |
| 10-OH diacid | — | 2340 | 2436 | — | — |
| $\Delta_1$ | — | — | — | — | — |
| $\Delta_2$ | — | — | — | — | — |
| 9,10, $\omega$-triOH | — | — | — | — | (2800 2752)[7] |
| $\Delta_1$ | — | — | — | — | 2718 |
| 9,10-diOH diacid | — | — | — | — | 2762 |
| $\Delta_1$ | — | — | — | — | — |

On the basis of the cutin acids now known, a few provisional empirical rules for cutin acid structure may be given: (1) All components of the cutin polymer are straight chain aliphatic compounds. No evidence has been found for branched, cyclic or aromatic compounds. (2) The principal cutin acids are $C_{16}$ and $C_{18}$ in chain length. (3) Odd chain lengths, in particular $C_{15}$ and $C_{17}$ are sometimes present in considerable amounts, the $C_{15}$ compounds being more frequent. (4) Most cutin components have a carboxyl function at one end and either a hydroxyl or another carboxyl function at the other. (5) Secondary hydroxyl groups are found only on the $C_9$ and/or $C_{10}$ positions. (6) Unsaturation does occur, although it does not seem to be very widespread.

Table 2 lists previously known cutin acids, those reported herein and those hitherto unreported but possible in the light of those already found. It is apparent that some 155 different acids are possible cutin constituents, ignoring anomalies and violations of the above rules. Excluded are compounds shorter than $C_{14}$ or longer than $C_{18}$ although traces of longer components were found in *Anemia phyllitides*.

*Mass Spectral Interpretation*

The MS of the known cutin acids and of similar hydroxy acids as their methyl esters, TMSi ethers have been already presented by us and others.[13,14] The MS of the same derivatives of the novel cutin acids could be readily interpreted in the light of the principles therein discussed.

In *Anemia phyllitides* a series of normal alcohols of $C_{28}$, $C_{30}$ and $C_{32}$ chain length were encountered. The identities of the alcohols were based on the retention indices (3152, 3350 and 3346, *n*-$C_{16}$OTMSi, 1950; programmed on SE-30) and mass spectra of the TMSi ethers.[17]

The MS of the methyl ester TMSi ethers of the novel cutin acids encountered in the course of this work are given in Figs. 3–11 and the rationale behind the structure assignments in Schemes 1–9.

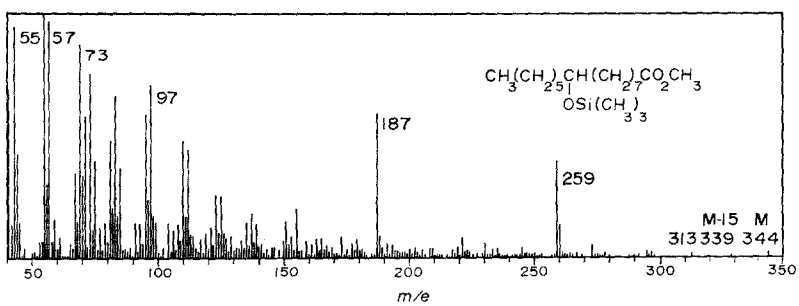



FIG. 3. MS OF 9-HYDROXYPENTADECANOIC ACID (METHYL ESTER, TMSi ETHER) FROM *Araucaria imbricata* PEAK 1, FIG. 2 AND SCHEME 1.

[13] P. CAPELLA and C. M. ZORZUT, *Analyt. Chem.* **40**, 1458 (1968).
[14] W. RICHTER and A. L. BURLINGAME, *Chem. Commun.* 1158 (1968).

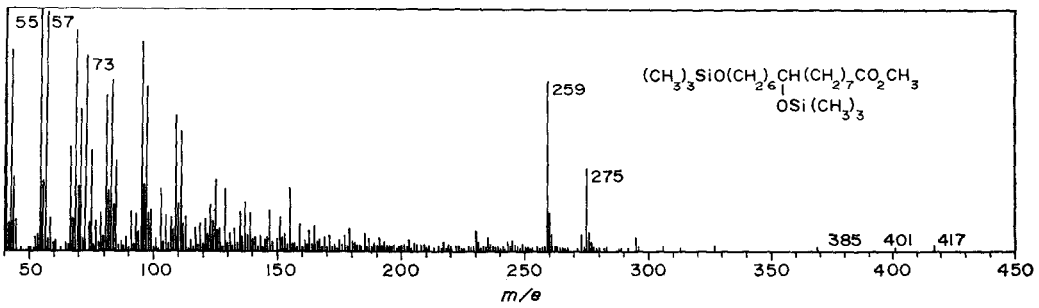



FIG. 4. MS OF 9,15-DIHYDROXYPENTADECANOIC ACID (METHYL ESTER BIS TMSi ETHER) FROM *Araucaria imbricata*, PEAK 5, FIG. 2 AND SCHEME 2.

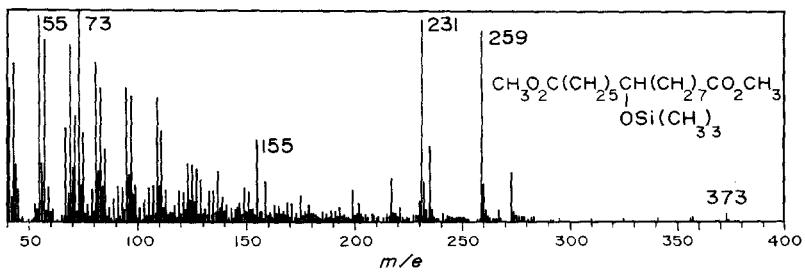



FIG. 5. MS OF 7-HYDROXYPENTADECAN-1,15-DIOIC ACID (DIMETHYL ESTER, TMSi ETHER) FROM *Araucaria imbricata*, FIG. 2, PEAK 4.

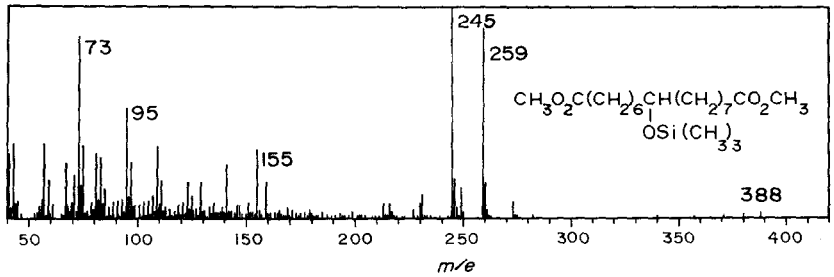



FIG. 6. MS OF 8-HYDROXYHEXADECAN-1,16-DIOIC ACID (DIMETHYL ESTER TMSi ETHER) FROM *Araucaria imbricata*, FIG. 2, PEAK 7.

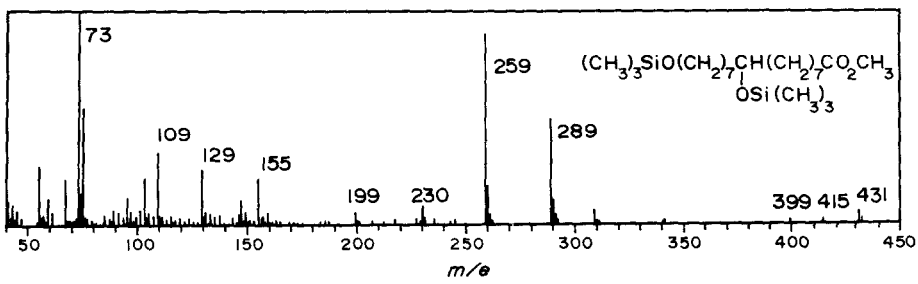



FIG. 7. MS OF 9,16-DIHYDROXYHEXADECANOIC ACID (DIMETHYL ESTER, BIS TMSi ETHER) FROM *Pinus sylvestris*.

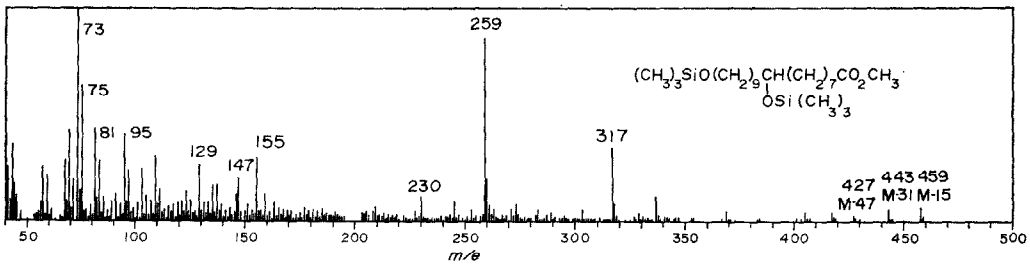



FIG. 8. MS OF 9,18-DIHYDROXYOCTADECANOIC ACID (METHYL ESTER, BIS TMSi ETHER) FROM *Pinus sylvestris*.

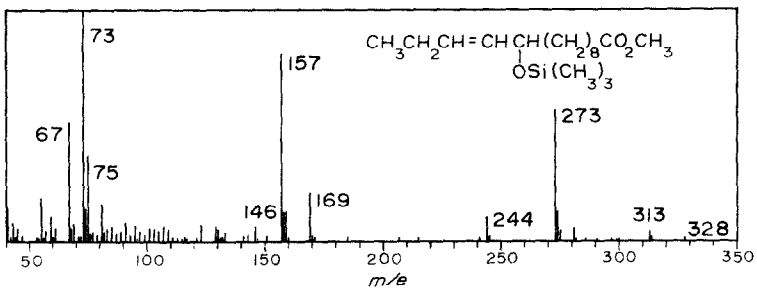



FIG. 9. MS OF 10-HYDROXYTETRADEC-(12)-ENOIC ACID (METHYL ESTER, TMSi ETHER) FROM *Welwitschia mirabilis*. THE POSITION OF THE DOUBLE BOND IN THE COMPOUND HAS NOT YET BEEN DEFINITELY ESTABLISHED.

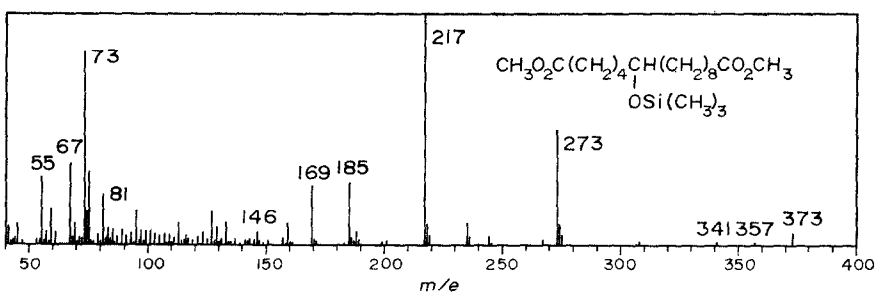



FIG. 10. MS OF 6-HYDROXYPENTADECAN-1,15-DIOIC ACID (DIMETHYL ESTER, TMSi ETHER) FROM *Gnetum gnemon*.

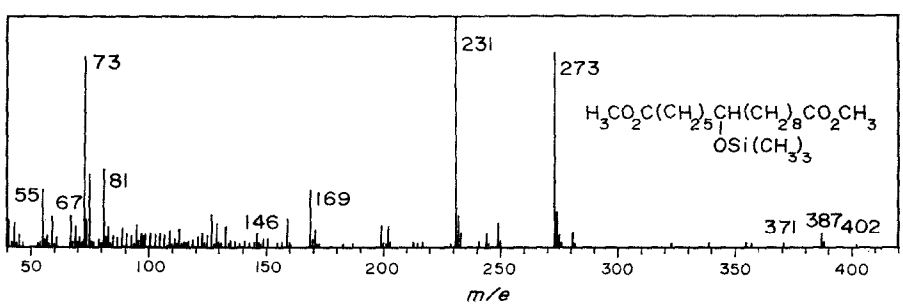
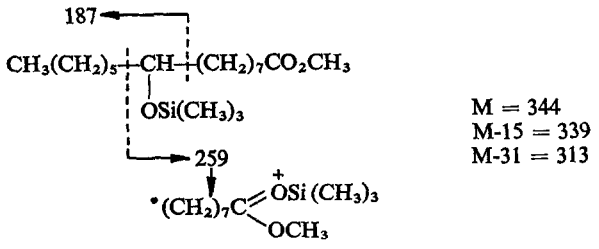
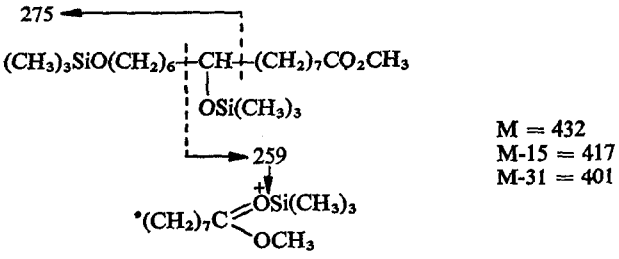



FIG. 11. MS OF 7-HYDROXYHEXADECAN-1,16-DIOIC ACID (DIMETHYL ESTER, TMSi ETHER) FROM *Gnetum gnemon*.



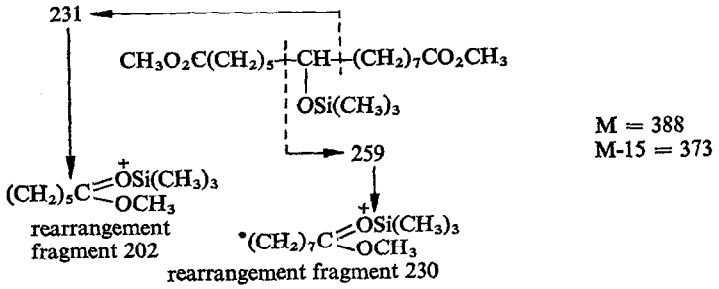

rearrangement fragment 230

SCHEME 1. MS FRAGMENTATION OF 9-HYDROXYPENTADECANOIC ACID, METHYL ESTER, TMSi ETHER FROM *Araucaria imbricata* (see Fig. 3).



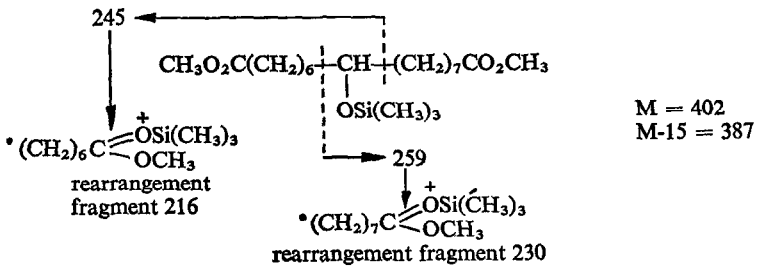

rearrangement fragment 230

SCHEME 2. MS FRAGMENTATION OF 9,15-DIHYDROXYPENTADECANOIC ACID, METHYL ESTER, BIS TMSi ETHER FROM *Araucaria imbricata* (see Fig. 4).



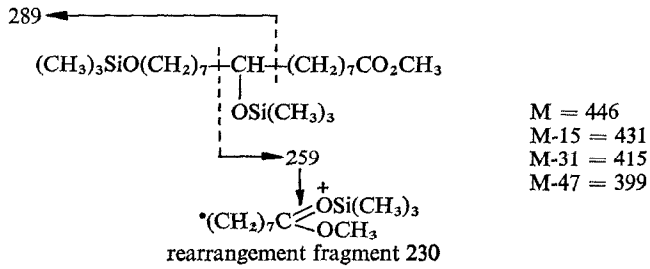

rearrangement fragment 230

SCHEME 3. MS FRAGMENTATION OF 7-HYDROXYPENTADECAN-1,15-DIOIC ACID, DIMETHYL ESTER, TMSi ETHER FROM *Araucaria imbricata* (see Fig. 5).

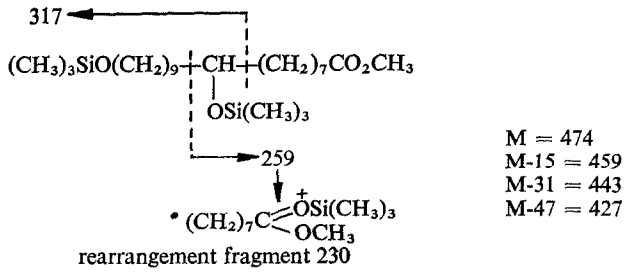



rearrangement fragment 230

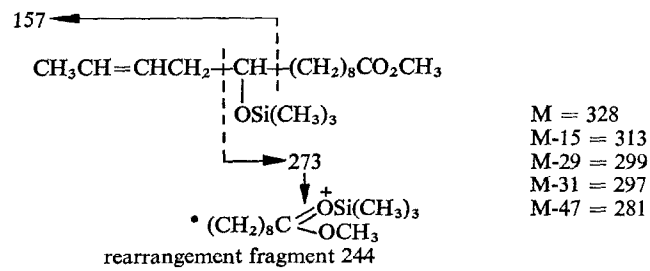
SCHEME 4. MS FRAGMENTATION OF 8-HYDROXYHEXADECAN-1,16-DIOIC ACID, DIMETHYL ESTER, TMSi ETHER FROM *Araucaria imbricata* (see Fig. 6).



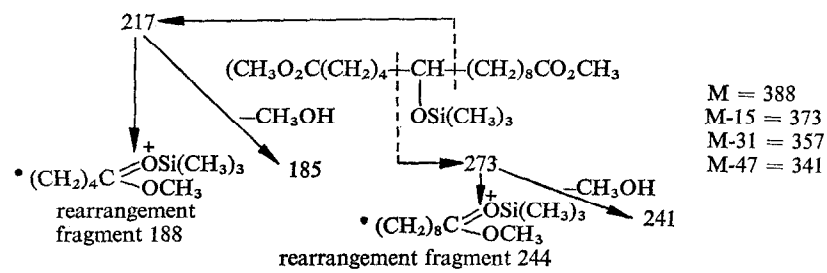

SCHEME 5. MS FRAGMENTATION OF 9,16-DIHYDROXYHEXADECANOIC ACID, METHYL ESTER, TMSi ETHER FROM *Pinus sylvestris* (see Fig. 7).



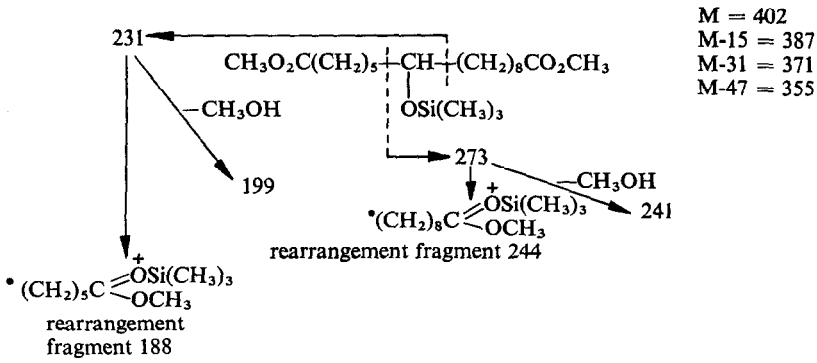

SCHEME 6. MS FRAGMENTATION OF 9,18-DIHYDROXYOCTADECANOIC ACID, METHYL ESTER, TMSi ETHER FROM *Pinus sylvestris* (see Fig. 8).




SCHEME 7. MS FRAGMENTATION OF 10-HYDROXYTETRADECANOIC ACID, METHYL ESTER, TMSi ETHER FROM *Welwitschia mirabilis* (see Fig. 9).




SCHEME 8. MS FRAGMENTATION OF 6-HYDROXYPENTADECAN-1,15-DIOIC ACID, DIMETHYL ESTER, TMSi ETHER FROM *Gnetum gnemon* (see Fig. 10).




SCHEME 9. MS FRAGMENTATION OF 7-HYDROXYHEXADECAN-1,16-DIOIC ACID, DIMETHYL ESTER, TMSi ETHER FROM *Gnetum gnemon* (see Fig. 11).

The principal cutin acid in *Pinus sylvestris* was found to be 9,16-dihydroxyhexadecanoic acid (Fig. 7). The discovery of this isomer, and other 9-hydroxylated compounds now casts doubt upon results in which identification of 10,16-dihydroxyhexadecanoic acid was based on TLC or GLC alone.[3–7] These two isomers could not be separated by TLC or GLC and only mass spectrometry could readily permit recognition of these compounds either pure or as mixtures.

Mixtures of the silyl ethers of 9- and 10-hydroxylated compounds were unresolvable with the GLC system used. However, the mass spectrum of each compound is characterized by about six ions and the major peaks in the spectra of the silyl ethers of 9- and 10-hydroxylated isomers differ by 14 mass units allowing a relatively simple evaluation of a composite GLC peak. Figure 12 shows the MS of a typical composite GLC peak (from *Ginkgo biloba*).

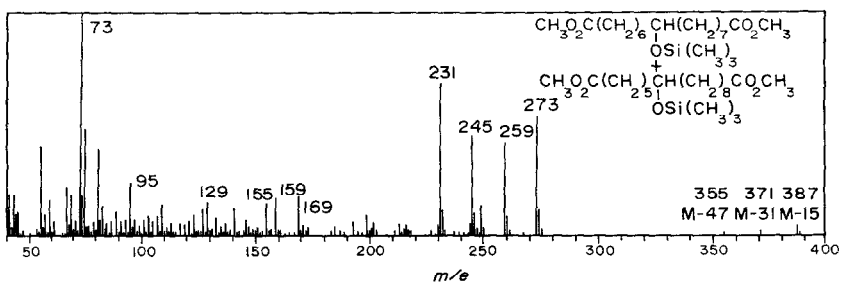



FIG. 12. MS OF A GLC PEAK CONTAINING A MIXTURE OF ISOMERIC CUTIN ACIDS (METHYL ESTERS, TMSi ETHERS) FROM *Ginkgo biloba*.

While 9,16-dihydroxyhexadecanoic acid methyl ester TMSi ether and 10,16-dihydroxyhexadecanoic acid methyl ester TMSi ether were not resolvable with the GLC system used, some slight separation could be detected by the following technique: A number of spectra were scanned through the major GLC peak of the cutin acid methyl ester TMSi ethers from *Araucaria imbricata* (at points A–E in Fig. 2). The partial spectra are shown in Fig. 13. The peaks $a_1$, $a_2$, $a_3$ come from 9,16-dihydroxyhexadecanoic acid methyl ester TMSi ether and $b_1$, $b_2$, $b_3$ from 10,16-hydroxyhexadecanoic acid methyl ester TMSi ether. By examination of the spectra, it is then clear that the 10,16-isomer slightly precedes the 9,16-isomer.

With the identification of these previously unreported cutin acids, we can clarify the identity of certain previously reported but unidentified compounds in apple fruit cutin.[8] Compound XXI[8] (5% of total cutin acids) was 6-hydroxypentadecan-1,15-dioic acid plus 13% of 7-hydroxypentadecan-1,15-dioic acid. The 10,16-dihydroxyhexadecanoic acid in this cutin (24% of total cutin) was found to contain about 13% of the 9,16-isomer. The peaks XXIX and XXX were found to be 9,10,18-trihydroxyoctadecenoic acid and *erythro*-9,10,18-trihydroxyoctadecanoic acid.[15]

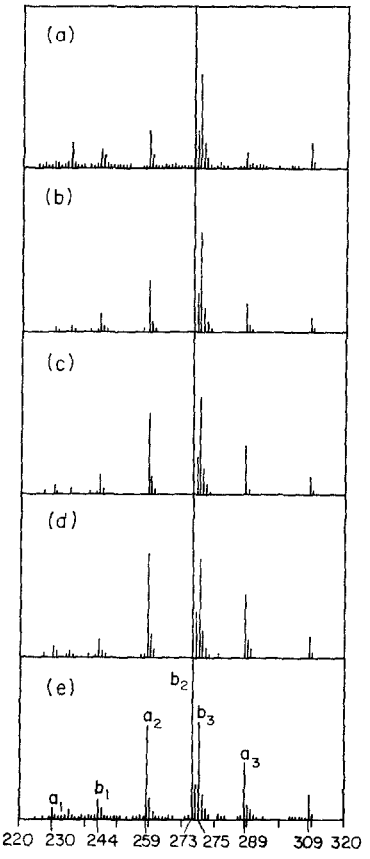



FIG. 13. PARTIAL MS TAKEN ON LARGE GLC PEAK IN Fig. 2. THIS SHOWS THE CHANGING COMPOSITION OF THIS COMPOSITE PEAK INDICATED BY THE RATIOS OF THE PEAK $a_1$, $a_2$, $a_3$ (FROM 9,16-DIHYDROXYHEXADECANOIC ACID (METHYL ESTERS, TMSi ETHERS)) AND $b_1$, $b_2$, $b_3$ (FROM THE 10,16-ISOMER).

## CONCLUSION

The GLC–MS technique has permitted the recognition of a number of novel cutin acids and a limited survey of the distribution of cutin acids in plants other than angiosperms. In the most primitive plants examined, a fern and a lycopod, the principal cutin acid was the monohydroxy acid, 16-hydroxyhexadecanoic. Species of gymnosperms and especially conifers had large amounts of 9,16-dihydroxyhexadecanoic acid. Species of Gnetophyta

[15] B. CROW, *B.Sc. Thesis*, Bristol University (1968).

had 10,16-dihydroxyhexade anoic acid as the principal cutin acid. A thorough chemotaxonomic survey of the cutin acids of plants other than angiosperms is indicated as being of potential value.

## EXPERIMENTAL

Between 0·5 and 2·5 g of leaves of each plant were cut into pieces approximately 2 × 2 cm (when size permitted). When the leaf was too small to be cut into pieces, then at least the edges were snipped off to allow the treating solutions to penetrate more easily. The pieces of leaf were briefly washed in MeOH to remove surface waxes and then soaked in 1·6% ammonium oxalate–0·4% oxalic acid solution at approx. 50° for 2–3 days. The separated cuticles were then washed thoroughly with distilled water and then soaked in $ZnCl_2$–conc. HCl (1:2) to remove cellulose. The cutin was then washed with distilled water, MeOH and MeOH–$CHCl_3$ and then air dried. No attempt was made to obtain all the purified cutin and small fragments were lost at each step and consequently cutin and cutin acid yields were not calculated. The isolated cutin was hydrolysed by refluxing overnight in 5% KOH in MeOH. The MeOH was evaporated and the residue acidified with aq. HCl and the ether soluble acids were then methylated with $CH_2N_2$ and bis(trimethylsilyl) acetamide added to form the trimethylsilyl ethers.

Retention indices[16] were determined by coinjecting the mixture of cutin acid methyl esters, TMSi ethers and a suitable mixture of *n*-alkanes and then temperature programming. GLC traces were determined on a Perkin–Elmer F-11 and GLC–MS was carried out on a LKB 9000 instrument. Details of procedures may be ound in ref. 8.

*Acknowledgements*—This work was supported by a grant from the National Aeronautics and Space Administration (NsG-101-61), GC–MS facilities were made available through a grant from the Science Research Council (B/SR/2398). We are extremely grateful to E. W. Curtis of the Glasgow Botanic Garden for assistance in obtaining plant material and to D. Brett of Bedford College for helpful discussions.

[16] H. VAN DEN DOOL and P. D. KRATZ, *J. Chromatog.* **11**, 463 (1963).
[17] A. G. SHARKEY, JR., R. A. FRIEDEL and S. H. LANGER, *Analyt. Chem.* **29**, 770 (1957).

*Key Word Index*—Gymnospermal; chemotaxonomy; cutin; GLC–MS; 9,16-dihydroxyhexadecanoic acid.