Phytochemistry, 1967, Vol. 6, pp. 727 to 733. Pergamon Press Ltd. Printed in England

TRITERPENOIDS FROM NEW ZEALAND PLANTS—II.*

THE TRITERPENE METHYL ETHERS OF *CORTADERIA TOETOE* ZOTOV

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(*Received* 16 *September* 1966)

Abstract—Application of gas–liquid chromatography and mass spectrometry has led to the identification of the three triterpene methyl ethers present in *Cortaderia toetoe* Zotov as arundoin, α-amyrin methyl ether and β-amyrin methyl ether.

INTRODUCTION

During an investigation of the alkane constituents of a number of native New Zealand plants,[1] strong i.r. absorption at 1104 cm^{-1} was observed in the alkane-containing fraction obtained from a specimen of "toetoe" grass, at that time designated *Arundo conspicua* Forst. f,[2] but subsequently identified[3] as *Cortaderia toetoe* Zotov.[4] Studies directed towards the isolation of the material responsible for the 1104 cm^{-1} absorption have resulted in the isolation of three triterpene methyl ethers. A preliminary report of this work has been given[5] in which one of the triterpene methyl ethers was identified as β-amyrin methyl ether (isosawamilletin[6]) (I), and another, designated arundoin, was erroneously concluded to be an oleanane derivative. The correct assignment of structure as 3β-methoxy-E:C*friedo*isohop-9(11)-ene (II) to arundoin has been made by Nishimoto *et al.*[7] who isolated this compound from the rhizomes of *Imperata cylindrica*,[8] direct comparisons of samples of arundoin from *C. toetoe* and *I. cylindrica* performed by both Dr. Natori and ourselves indisputably proving their identity. The present paper describes the full characterizations of the triterpene methyl ethers from *C. toetoe* and records what would appear to be the first instance of the isolation of α-amyrin methyl ether (III) from natural sources.

DISCUSSION

Application of alumina column chromatography, employing light petroleum (b.p. 40–60°) as eluant to the total light petroleum extractives from the unmacerated green leaves of a

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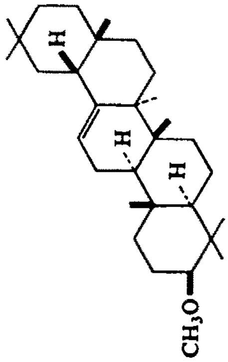
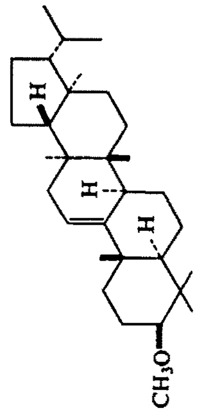


(I)



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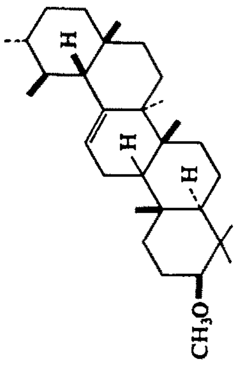

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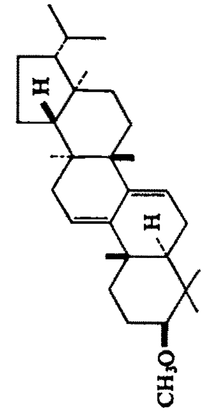

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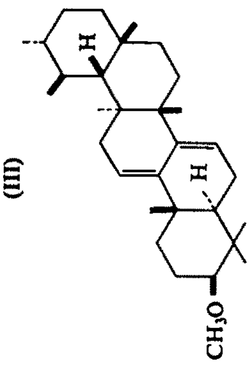

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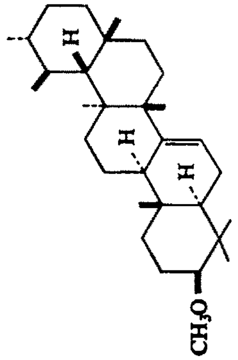

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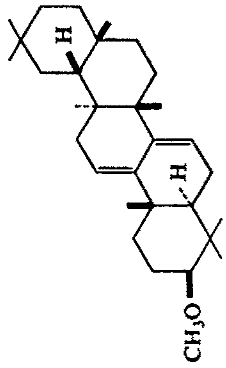

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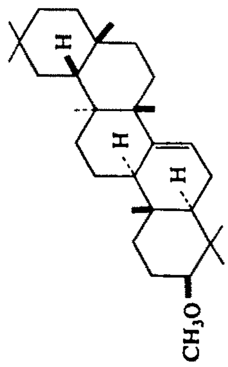

(VIII)

Cortaderia species collected at Plimmerton, New Zealand, and identified[3] as *C. toetoe* Zotov, gave, after initial elution of alkanes, a crystalline fraction which was shown to be the material responsible for the 1104 cm^{-1} absorption in the i.r. spectrum of the total light petroleum extractives. Although this material appeared homogeneous on thin-layer chromatography employing a variety of adsorbants and solvent systems, application of gas–liquid chromatography, using 0·5% Apiezon L supported on Gas-Chrom Z of 100–120 mesh as stationary phase at 240°, revealed the presence of three well-resolved peaks. Addition of 5α-cholestane (retention time = 1) to the mixture to serve as internal standard, showed the relative retention times of the three components to be 2·79, 3·18 and 4·28 (major peak—arundoin). Further GLC employing 1·5% SE-30 columns at 240° showed that the retention times of the three components relative to 5α-cholestane were 2·44, 2·72 and 3·21 (arundoin) respectively.

From detailed studies of the GLC behaviour of authentic triterpene methyl ethers published elsewhere[9] these retention values immediately suggested that in addition to arundoin, *C. toetoe* could contain the methyl ether of α-amyrin (retention times of 3·20 ± 0·05 on Apiezon L and 2·73 ± 0·05 on SE-30[9]) and one or more of the methyl ethers of germanicol, δ-amyrin, β-amyrin, taraxerol or multiflorenol (retention times of 2·79 ± 0·05 on Apiezon L and 2·49 ± 0·05 on SE-30[9]), which have been shown to be indistinguishable on GLC employing Apiezon L and SE-30 as stationary phases under the conditions presently employed.

Further alumina column chromatography of the mixture of triterpene methyl ethers from *C. toetoe* was successful in providing pure arundoin as the first fraction to be eluted, whilst the later fractions became progressively enriched with the components having the GLC retention times of 2·79 and 3·18 on the Apiezon L column. In order to secure these components separately, resort was made to preparative GLC, and each component was then subjected to mass spectroscopy in order to use the characteristic mass spectral fragmentations of triterpenes[9–12] as a means of identification.

β-Amyrin Methyl Ether (*I*)

The component of relative retention time 2·79 collected by preparative GLC using an Apiezon L column had m.p. 246–248° (lit.[6, 13] for β-amyrin methyl ether 247–248°) and showed no m.p. depression on admixture with an authentic sample prepared from β-amyrin by adaptation[9] of the method of Morice and Simpson.[13]

The i.r. spectra in KCl disc were also identical, but, in order to ascertain that no discernible amounts of other triterpene methyl ethers derived from the oleanane skeleton were contaminating the material, detailed comparison of its mass spectrum was made with the mass spectrum of authentic β-amyrin methyl ether—both being obtained with a direct inlet system (Fig. 1*a*). The absence of any ion of m/e 234, the expected[11] base peak in the mass spectrum of multiflorenol methyl ether, in both spectra showed the absence of the latter as a contaminant of the β-amyrin methyl ether in *C. toetoe*. Similarly the complete identity of the mass spectra of the natural and authentic samples in the 203–205 mass unit region rules out the possibility of the presence of any appreciable quantities of taraxerol methyl ether (base peak m/e 204[9, 11]), germanicol methyl ether (prominent peaks at m/e 203, 204 205,[10]), or δ-amyrin methyl ether (base peak m/e 205[11]) as contaminants of the β-amyrin methyl ether from *C. toetoe*.

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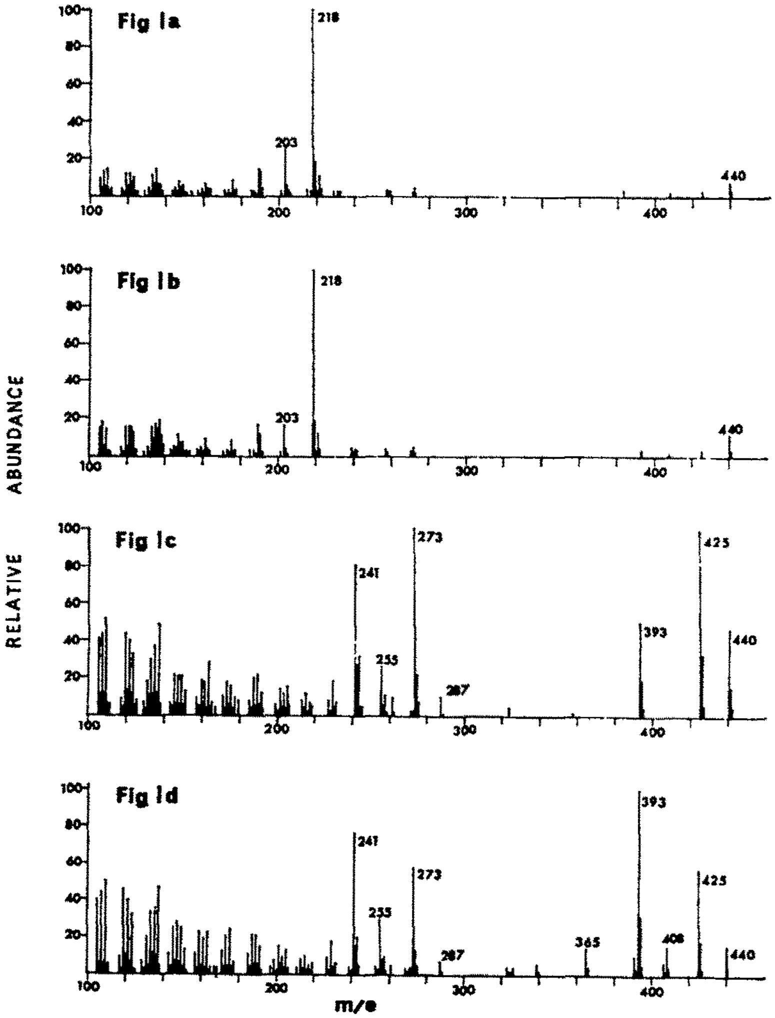

FIG. 1. LINE DIAGRAMS OF THE MASS SPECTRA OF TRITERPENE METHYL ETHERS.
(*a*) β-Amyrin methyl ether using direct inlet system; (*b*) α-amyrin methyl ether using direct inlet system; (*c*) arundoin using direct inlet system; (*d*) arundoin using heated inlet system.

α-Amyrin Methyl Ether (III)

The material of relative retention time 3·18 collected by preparative GLC using an Apiezon L column had m.p. 221–223° (reported[13] for α-amyrin methyl ether 221–222°) and showed no m.p. depression on admixture with an authentic sample prepared from α-amyrin by adaptation[9] of the method of Morice and Simpson.[13] The i.r. spectra in KCl disc of the two specimens were identical as were their mass spectra—both determined with a direct inlet system (Fig. 1*b*).

Arundoin (II)

Pure arundoin (as evidenced by analytical GLC) had $[\alpha]_D -5{\cdot}7°$ (in $CHCl_3$) and was found to exhibit dimorphism, the two forms having m.p's of 235–237° and 271–273° respectively. That polymorphism may be of not rare occurrence among triterpene methyl ethers is suggested by the fact that a specimen of taraxerol methyl ether synthesized in our laboratories exhibited dimorphism with a specimen of natural occurrence (sawamilletin) supplied by Dr. Abe, the two samples having m.p's of 270–273° and 276–278° respectively, but having identical i.r. spectra in CCl_4.

Originally[5] we reported that the trisubstituted double bond of arundoin was not isomerized by the action of acid, but re-investigation has shown that acid isomerizes arundoin into a compound of m.p. 223–224° and $[\alpha]_D + 30°$ ($CHCl_3$), in which the double bond is tetrasubstituted, in agreement with the work of the Japanese group.[7] Re-investigation of the NMR spectrum of arundoin and comparison with the NMR spectra of other triterpene methyl ethers has shown that the strong singlet at 6·62 τ (OCH_3), which was previously considered[5] to have an intensity of 4 protons, has in fact an intensity of 3 protons, while the hydrogen atom in the 3α-position gives rise to a low diffuse absorption centred near 7·4τ and *ca.* 30 c/s broad.

The mass spectra of arundoin obtained with a direct inlet system and a heated inlet system (Fig. 1*c* and Fig. 1*d*, respectively) showed certain points of difference. Thus when a heated inlet system was employed a prominent peak was present at m/e 408 (parent minus methanol) with an observed metastable ion at 378·3 (calculated for m/e 440→408, 378·3). This peak was not discernible in the mass spectrum obtained with a direct inlet system. Again the ions at m/e 393, 255 and 241 are more abundant than the ions from which they are derived through loss of methanol (now established[9] to be a characteristic fragmentation of triterpene methyl ethers) with the heated inlet system and less abundant than the ions from which they are derived with the direct inlet system. Moreover the presence in the mass spectrum of arundoin determined with the heated inlet system (unavailable to us earlier[5]) of an ion at m/e 365, obviously derived by loss of an isopropyl group (43 mass units) from the ion m/e 408 as apparent by the presence of a metastable ion at 326·5 (calculated 326·5), provides confirmatory evidence for the isopropyl group in arundoin.

In the present work the 7,9(11)-diene (V) was prepared from bauerenol methyl ether (VI) and the 7,9(11)-diene (VII) was prepared from multiflorenol methyl ether (VIII), and the non-identity of both dienes with the 7,9(11)-diene (IV) from arundoin confirmed.

EXPERIMENTAL

Materials and Methods

Melting points were determined with a Kofler block. Optical rotations were measured in $CHCl_3$ solution in a 10 cm cell with a Bellingham Stanley "Pepol-60" instrument. Mass spectra were determined with an A.E.I. M.S.9 double-focusing mass spectrometer.[9] Analytical GLC was conducted with a standard Pye Panchromatograph,[3, 9] and preparative GLC on an Aerograph A-700 (Autoprep) instrument (Wilkens Instrument and Research Inc., Walnut Creek, California) fitted with standard copper tubes *ca.* 2 m × 6 mm containing 1·0% Apiezon L supported on Gas-Chrom Z (Applied Science Laboratories Inc., State College, Pennsylvania, U.S.A.) of 100–120 mesh. Helium was employed as carrier gas. The detector was of the thermal conductivity type with a Honeywell Brown (Newhouse, Lanarkshire, Scotland) pen recorder with sensitivity 0–10 mV. Column temperature 240°; detector temperature 280°; helium gas flow rate 60 ml/min at outlet; filament current 150 mA.

Isolation of Triterpene Methyl Ethers

Fresh unmacerated green leaves of *C. toetoe* Zotov (3 × 25 kg) were cut into 25-cm lengths and immersed in light petroleum of b.p. 40–60° for 16 hr at room temperature. Decantation and removal of solvent under reduced pressure afforded a pale yellow wax (275 g).

The light petroleum extractives (200 g) were taken up in light petroleum, b.p. 40–60°, and filtered through alumina (Brockmann, Grade V) with further light petroleum, until no further elution occurred. The initial eluates contained alkanes but subsequent fractions yielded crystalline material (4·5 g), which was rechromatographed over basic alumina (Woelm grade I) using light petroleum, b.p. 40–60°. The initial eluates afforded pure arundoin (II) (2·15 g), but subsequent fractions were shown by analytical GLC to contain at least two other components which were separated by preparative GLC on 1 % Apiezon L as stationary phase.

Automatic direct injections (15–20 μl) of a solution of the mixed triterpene methyl ethers (80 mg) in chloroform (0·25 ml) were made on to the column through a silicone-rubber "blind hole" stopper. The fractions corresponding to the three well-resolved peaks on the trace were collected as they eluted from the column in capillary glass tubes. After 9 cycles were obtained fraction A (relative retention time 2·79)—12 mg; fraction B (relative retention time 3·18)—5 mg; and arundoin (relative retention time 4·28)—20 mg.

Fraction A had m.p. 246–248°, which was undepressed on admixture with authentic β-amyrin methyl ether (I). Infrared (KCl disc) and mass spectra of the two specimens were identical.

Fraction B had m.p. 221–223°, which was undepressed on admixture with authentic α-amyrin methyl ether (III). Infra-red (KCl disc) and mass spectra of the two specimens were identical.

Arundoin (II)

Arundoin was found to exist in two forms (m.p. 235–237° and m.p. 271–273°)—which showed identical GLC retention times on 0·5 % Apiezon L at 240° and i.r. spectra in CCl_4. The higher melting form was converted into the lower one on crystallization from ethyl acetate with addition of a seed crystal of the lower melting form to induce crystallization. A specimen ($[\alpha]_D = -5{\cdot}7°$), m.p. 235–237°, gave: (Found: C, 84·6; H, 11·9. $C_{31}H_{52}O$ required: C, 84·5; H, 11·9 %).

3β-Methoxy D:C-friedo-*oleana*-7,9(11)-*diene (VII)*

A suspension of multiflorenol methyl ether (VIII),[9] 100 mg, in glacial acetic acid (25 ml) and SeO_2 (100 mg) in acetic acid (2·6 ml), was heated at 100° for 1 hr. After cooling and filtering the mixture was diluted with water and the precipitated solid collected and dried. It was taken up in light petroleum (50 ml) and filtered through a column of alumina (Woelm, basic, 3 g) employing a further 150 ml of light petroleum to complete the elution of crystalline *3β-methoxy D:C*-friedo-*oleana*-7,9(11)-*diene* of m.p. 226–228°. Found: C, 84·7; H, 11·5. $C_{31}H_{50}O$ required: C, 84·8; H, 11·5 %. λ_{max} 232, 239 nm (ϵ 16,100, 16,800) with shoulder 247.5 nm (ϵ 9500) in hexane.

3β-Methoxy D:C-friedo-*ursa*-7,9(11)-*diene (V)*

To SeO_2 (150 mg) in 96 % acetic acid (2·5 ml) was added a suspension of bauerenol methyl ether (VI),[9] 150 mg, in glacial acetic acid (25 ml) and the mixture heated at 100° for 1 hr. After cooling, filtering and dilution with water, a crystalline solid separated. This was washed with water, dissolved in light petroleum, b.p. 40–60° (50 ml), and filtered through a column of alumina (Woelm, basic, 3 g). Elution of the column with further light petroleum (100 ml) and combination of the total eluates afforded, after removal of solvent, *3β-methoxy-D:C-*friedo-*ursa-*7,9(11)-*diene* (100 mg) which on recrystallization from ethyl acetate had m.p. 182–183°; λ_{max} 232, 239·5 nm (ϵ 16,600, 17,400) and shoulder 248 nm (ϵ 10,200) in hexane. Found: C, 84·2; H, 11·5. $C_{31}H_{50}O$ required: C, 84·8; H, 11·5%.

Acknowledgements—One of us (M. M.-S.) wishes to acknowledge the receipt of a generous travel grant from the Wellcome Trust. He also wishes cordially to thank Professors S. N. Slater and J. F. Duncan of Victoria University of Wellington for kind permission to use facilities in their laboratories for large-scale extraction of *C. toetoe*.