Phytochemistry, 1976, Vol. 15, pp. 1933–1935. Pergamon Press. Printed in England.

TRITERPENE METHYL ETHERS OF *CHIONOCHLOA* (GRAMINEAE)

GRAEME B. RUSSELL*, HENRY E. CONNOR† and ANDREW W. PURDIE†
*Applied Biochemistry Division, DSIR, Palmerston North, New Zealand; †Botany Division, DSIR, Christchurch, New Zealand

(*Revised received* 11 *March* 1976)

Key Word Index—*Chionochloa*; Gramineae; epicuticular wax; triterpene methyl ethers; arundoin; miliacin; lupeol methyl ether; β-amyrin methyl ether; parkeol methyl ether; cycloartenol methyl ether.

Abstract—The leaf wax of twenty species of *Chionochloa* was examined for triterpene methyl ethers; twelve species gave positive yields. The known pentacyclic methyl ethers arundoin, miliacin, lupeol methyl ether and β-amyrin methyl ether were identified and the methyl ethers of the tetracyclic alcohols, cycloartenol and parkeol are reported as new natural products. Arundoin and miliacin occur in many species while the remaining compounds may be suitable chemotaxonomic markers.

INTRODUCTION

Triterpene methyl ethers occur widely in the Gramineae having been isolated from 14 Tribes [1]. Since these compounds do not occur to any great extent in other families they are considered to be the most significant triterpenoid characteristic of the Gramineae. As yet, however, their distribution has not contributed significantly to taxonomy as the same compounds are widely distributed, and in no one genus has a sufficiently large number of species been examined to test their suitability.

Chionochloa, a genus with nineteen species in New Zealand, one in the Subantarctic Islands and one in Australia [2], is physiognomically important in the mountain lands of New Zealand. Since there are still some taxonomic difficulties in the New Zealand species, a study of the triterpene methyl ether content of the leaf waxes was undertaken to help resolve some problems. From 12 of the 20 species examined we have isolated and identified six triterpene methyl ethers. Two of these are new natural products being methyl ethers of the tetracyclic alcohols cycloartenol and parkeol.

RESULTS AND DISCUSSION

Leaves of species of *Chionochloa* collected from geographically, altitudinally and ecologically different regions in New Zealand, were extracted and the epicuticular waxes fractionated as described in Experimental. Where necessary triterpene methyl ethers were isolated by preparative GLC.

The known pentacyclic triterpene methyl ethers arundoin (**1**), miliacin (**2**), lupeol methyl ether (**3**) and β-amyrin methyl ether (**4**) were crystallized and identified from the MS fragmentation patterns [3], NMR, GLC [1], mp and optical rotations. Two triterpene methyl ethers of low melting point were recognised as tetracyclic derivatives from the MS which showed no strong characteristic peaks other than loss of methyl and methanol from the molecular ions. The NMR spectra showed the presence of a lanosterol-type side chain in both compounds with 3 proton singlets at δ 1.71 and 1.64. One of these compounds was cycloartenol methyl ether (**5**) which gave signals in the NMR at δ 0.59 and 0.33 (J = 4 Hz) due to the cyclopropane moiety and a triplet at δ 5.14–5.00 (J = 6 Hz) due to a vinylic proton. This compound is a new natural product although it has been previously synthesized [4]; the physical data were consistent. The other tetracyclic compound was identified as parkeol methyl ether (**6**). The methyl region of the NMR spectrum was consistent with that published for parkeol benzoate [5] giving 3 proton signals at δ 1.06 (C-32) 0.98 (C-30) 0.89 (*d*, J = 6 Hz, C-21) 0.82 (C-31) 0.76 (C-19) 0.66 (C-18) 1.70, 1.63 (C-26, C-27) and one proton signals at δ 5.21 and 5.06. Parkeol methyl

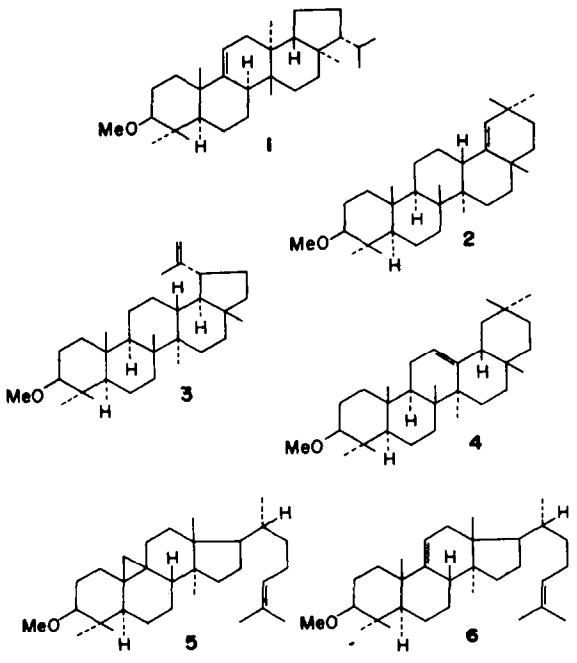

Table 1. Triterpene methyl ethers in species of *Chionochloa*

	Arundoin	Miliacin	Lupeol methyl ether	β-Amyrin methyl ether	Parkeol methyl ether	Cycloartenol methyl ether
C. acicularis Zotov						+
C. antarctica (Hook.f.) Zotov	+				+	
C. australis (Buch.) Zotov*			+			
C. bromoides (Hook.f.) Zotov	+					
C. cheesemanii (Hack. ex Cheeseman) Zotov†	+		+			
C. crassiuscula (Kirk) Zotov†	+	+				
C. flavescens Zotov*	+	+	+			
C. oreophila (Petrie) Zotov*					+	
var. *elata* (Petrie) Zotov		+				
C. pallens Zotov†	+	+			+	+
C. pungens (Cheeseman) Zotov	+	+				
C. rigida (Raoul) Zotov*†	+			+		
C. rubra Zotov*†	+	+			+	+
C. beddiei Zotov	No triterpene methyl ethers					
C. conspicua (Forst.f.) Zotov						
C. flavicans Zotov						
C. frigida (Vickery) Conert						
C. juncea Zotov						
C. macra Zotov						
C. spiralis Zotov						
C. teretifolia (Petrie) Zotov						

* Triterpene methyl ethers are not found in all populations.
† Triterpene methyl ether complement differs between populations.

ether was reduced to give the 24-25-dihydro compound and this was identical with an authentic sample prepared from cycloartenol methyl ether [6].

The distribution of the triterpene methyl ethers found in 20 species of *Chionochloa* is given in Table 1. Only *C. ovata* (Buch.) Zotov was not examined. Eight species yielded no triterpene methyl ethers, and in *C. flavescens* and *C. rigida* triterpenoids occurred infrequently. Arundoin was the most common methyl ether, occurring in nine species; fern-9(11)-ene derivatives have proved to occur widely in the Gramineae [1]. Miliacin was present in six taxa and lupeol and β-amyrin methyl ethers showed occasional occurrence. Tetracyclic triterpenes are uncommon in the Gramineae [1], their occurrences being confined to cycloartenol, which has been identified in maize [7,8] and rice [9,10], and 24-methylenecycloartanol and β-cycloorystenol, both also from *Oryza sativa* [11,12]. Cycloartenol methyl ether was isolated in reasonable yields from *C. acicularis*, where it was the only triterpene methyl ether obtained and from some populations of *C. rubra* and *C. pallens*. These latter two species display the greatest versatility in triterpene methyl ether synthesis and samples from different localities gave extracts where the kind of methyl ether(s) varied. Parkeol methyl ether was isolated from four species: *C. antarctica* and *C. rubra* where it was the major triterpene occurring with arundoin; from one population of *C. pallens* where it co-occurred with arundoin and cycloartenol methyl ether; from one population of *C. oreophila*. Parkeol is a triterpene of very infrequent occurrence having been isolated only from *Butyrospermum parkii* [13] and yeast extract [5].

Both cycloartenol and parkeol arise by cyclisation of squalene-2,3-oxide and cycloartenol can be further metabolised to produce the typical phytosterols. The synthesis of the methyl ethers would occur in plants having genes controlling methylation. However, the fact that other sterol methyl ethers have not been found might suggest that methylation can only occur at the triterpene stage of synthesis.

A complete analysis of *Chionochloa* for triterpene methyl ethers has provided some suitable biochemical markers for studies in taxonomy and genecology. These data will be discussed in a subsequent publication.

EXPERIMENTAL

GLC conditions were: glass column, 165 cm × 4 mm, 0.75% Apiezon L on Chromosorb W, temp. 240°, N_2 40 ml/min, retention times were relative to 5α-cholestane. Rotations were measured in $CHCl_3$ at 20°. NMR spectra were measured in $CDCl_3$ at 60 MHz.

Isolation of surface waxes. Dry clean leaves and sheaths from 161 collections, (specimens at CHR), were cut to length and immersed in redistilled petrol (bp 40–60°) for 16 hr at room temp., and concentrated wax obtained from the soln by removal of solvent under vacuum.

Isolation of triterpene methyl ethers. The total petrol extracts from each collection were chromatographed on neutral alumina (Woelm grade 1) with petrol and petrol–C_6H_6 and the triterpene methyl ether fraction was further separated by preparative GLC where necessary. Pure materials were recrystallized from EtAc–MeOH.

Arundoin. Mp 232–3°. $[\alpha]_D$ – 2.5° (*c*, 1.25), (lit. [14]: 235–7°, – 5.3°). GLC retention time, 4.30. Identical with an authentic sample [15] by MS, NMR, mmp.

Miliacin. Mp 280–2°. $[\alpha]_D$ + 22° (*c*, 0.88), (lit. [16]: 285–6°, + 16°). GLC retention time 2.84. MS: *m/e* 440, 425, 218, 204, 189, 177. NMR: δ 4.86 (1H), 3.36 (3H), 2.66 (*q*, 1H), 1.06 (3H), 1.02 (3H), 0.93 (9H), 0.87 (3H), 0.73 (6H).

Lupeol methyl ether. Mp 249–51°. $[\alpha]_D$ + 45° (*c*, 1), (lit. [17]: 250°, + 36°). GLC retention time, 3.15. MS: *m/e* 440, 425, 408, 393, 330, 329, 221, 218, 204, 203, 191, 190, 189. NMR: δ 4.68 (*d*, *J* = 2 Hz, 1H), 4.57 (*d*, *J* = 2 Hz, 1H), 3.35 (3H), 2.63 (*q*, 1H), 1.72 (3H), 1.05 (3H), 0.97 (6H), 0.85 (3H), 0.80 (3H), 0.75 (3H).

β-Amyrin methyl ether. Mp 249–52°. $[\alpha]_D$ + 103° (*c*, 0.44), (lit. [14]: 247–8°, + 98°). GLC retention time, 2.77. Identical with an authentic sample [15] by MS, NMR, mmp.

Parkeol methyl ether. Mp 140–2°. $[\alpha]_D$ + 89° (*c*, 0.73). GLC retention time, 2.92. MS: *m/e* 440, 425, 408, 393, 327, 189, 187, 175, 173. NMR: δ 5.21 (1H), 5.06 (1H), 3.38 (3H), 2.67 (*q*, 1H), 1.70 (3H), 1.63 (3H), 1.06 (3H), 0.98 (3H), 0.89 (*d*, *J* = 6 Hz, 3H), 0.82 (3H), 0.76 (3H), 0.66 (3H). Parkeol methyl ether in ethyl acetate was hydrogenated in the presence of platinum oxide to give 24,25-dihydroparkeol methyl ether, Mp. 141–2°, $[\alpha]_D$ + 91° (*c*, 0.56), identical to a sample prepared from cycloartenol methyl ether.

Cycloartenol methyl ether. Mp 118–20°. $[\alpha]_D$ + 66° (*c*, 1.8), lit. [4]: 120–22°, + 54°). GLC retention time, 3.24. MS: *m/e* 440, 425, 408, 393, 365, 339, 286, 203, 187. NMR: δ 5.07 (*t*, 1H), 3.38 (3H), 2.68 (*q*, 1H), 1.71 (3H), 1.64 (3H), 0.98 (7.5H), 0.91 (4.5H), 0.82 (3H), 0.59 (*d*, *J* = 4 Hz, 1H), 0.33 (*d*, *J* = 4 Hz, 1H). Cycloartenol methyl ether (100 mg) in EtOAc was hydrogenated in the presence of platinum oxide to give cycloartanol methyl ether, mp 136–8°. This was dissolved in $CHCl_3$ and saturated with dry HCl for 6 hr. After removal of the solvent, CrO_3 (0.3 mg) in 90% HOAc (9 ml) was added and the mixture heated for 15 min, poured into H_2O and extracted with $CHCl_3$. The residue was eluted from a column of alumina

with petrol–C_6H_6 (95:5) to give 24,25-dihydroparkeol methyl ether (mp, mmp, MS, NMR, $[\alpha]_D$).

Two further triterpene methyl ethers have been isolated but as yet they have not been identified.

C. bromoides. Compound A, mp 185–7° $[\alpha]_D$ + 67° (c, 2). GLC retention time 3.30.

C. cheesemanii. Compound B, mp 238–41° $[\alpha]_D$ + 74.5° (c, 0.94). GLC retention time 6.45.

REFERENCES

1. Ohmoto, T., Ikuse, M. and Natori, S. (1970) *Phytochemistry* **9**, 2137.
2. Zotov, V. D. (1963) *N.Z. J. Botany* **1**, 78.
3. Budzikiewicz, H., Wilson, J. M. and Djerassi, Carl (1963) *J. Am. Chem. Soc.* **85**, 3688.
4. Ritchie, E., Senior, R. G. and Taylor, W. C. (1969) *Australian J. Chem.* **22**, 2371.
5. Barton, D. H. R., Kempe, U. M. and Widdowson, D. A. (1972) *J. Chem. Soc. (Perkin)* 513.
6. Bentley, H. R., Henry, J. A., Irvine, D. S. and Spring, F. S. (1953) *J. Chem. Soc.* 3673.
7. Kemp, R. J., Hamman, A. S. A., Goad, L. J. and Goodwin, T. W. (1967) *Phytochemistry* **6**, 447.
8. Kemp, R. J. and Mercer, E. I. (1968) *Biochem. J.* **110**, 111.
9. Ohta, G. and Shimizu, M. (1957) *Chem. Pharm. Bull. Tokyo* **5**, 40.
10. Shimizu, M., Uchimaru, F. and Ohta, G. (1964) *Chem. Pharm. Bull. Tokyo* **12**, 74.
11. Ohta, G. and Shimizu, M. (1958) *Chem. Pharm. Bull. Tokyo* **6**, 325.
12. Yoshida, S., Takasaki, R. and Sueyoshi, H. (1956) *J. Pharm. Soc. Japan.* **76**, 1335.
13. Dawson, M. C., Halsall, T. G., Jones, E. R. H. and Robins, P. A. (1953) *J. Chem. Soc.* 586.
14. Bryce, T. A., Eglinton, G., Hamilton, R. J., Martin-Smith, M. and Subramanian, G. (1967) *Phytochemistry* **6**, 727.
15. Martin-Smith, M., Ahmed, S. and Connor, H. E. (1971) *Phytochemistry* **10**, 2167.
16. Sorkina, T. I., Segal, G. M., Belova, S. M., Chernogubova, I. P. and Torgov, I. V. (1968) *Izv. Akad. Nauk. SSSR, Ser. Khim.* **5**, 1127.
17. Ohmoto, T. and Natori, S. (1969) *Chem. Commun.* 601.