*Acknowledgements*—We thank Prof. M. Ladero and Dr. C. Valle (Botany Dept.) for the localization and identification of the plant; Prof. J. de Pascual Teresa (Faculty of Chemistry, Salamanca) for the facilities kindly offered at his laboratory and Prof. A. G. González (La Laguna, Spain) for the MS. Financial support came from the 'Junta de Castilla y Leon'.

REFERENCES

1. Villar, A., Calduch, M. L. and Zafra-Polo, M. C. (1983) *Ars Pharm.* **24**, 149.
2. Kelsey, R. G. and Shafizadeh, F. (1979) *Phytochemistry* **18**, 1591.
3. Seaman, F. C. (1982) *Bot. Rev.* **48**, 121.
4. Hegnauer, R. (1964) *Chemotaxonomie der Pflanzen*, Vol. III, p. 470. Birkhäuser, Basel.
5. Kawatani, T. and Vodopivec, S. (1956) *J. Pharm. Soc. Japan* **76**, 1214.
6. Fischer, N. H., Olivier, E. J. and Fischer, H. D. (1979) in *Progress in the Chemistry of Natural Products*, Vol. 39, p. 164. Springer, New York.
7. Akyev, B., Kasymov, S. Z. and Sidyakin, G. P. (1972) *Khim. Prir. Soedin.* 733.
8. Tarasov, V. A., Kasymov, S. Z. and Sidyakin, G. P. (1973) *Khim. Prir. Soedin.* 676.
9. González, A. G., Bermejo, J., Mansilla, H., Massanet, G. M., Cabrera, I., Amaro, J. M. and Galindo, A. (1977) *Phytochemistry* **16**, 1836.
10. Yoshioka, H., Mabry, T. J. and Timmermann, B. N. (1973) *Sesquiterpene Lactones: Chemistry, NMR and Plant Distribution.* University of Tokyo Press, Tokyo.
11. Legrand, M. and Rougier, M. J. (1977) in *Stereochemistry: Fundamental and Methods* (Kagan, H. B., ed.) Vol. 2, p. 123. Georg Thieme, Stuttgart.

*Phytochemistry*, Vol. 25, No. 7, pp. 1759–1761, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

# IDENTIFICATION OF MOLLIC ACID α-L-ARABINOSIDE, A 1α-HYDROXYCYCLOARTENOID FROM *COMBRETUM MOLLE* LEAVES

C. B. ROGERS* and I. THEVAN

Department of Chemistry, University of Durban-Westville, Private Bag X 54001, Durban 4000, Republic of South Africa

(*Received* 11 *November* 1985)

**Key Word Index**—*Combretum molle*; Combretaceae; 1α-hydroxycycloartenoids; mollic acid α-L-arabinopyranoside.

**Abstract**—A further novel 1α-hydroxycycloartane glycoside, mollic acid α-L-arabinoside, has been identified in the leaves of *Combretum molle*.

## INTRODUCTION

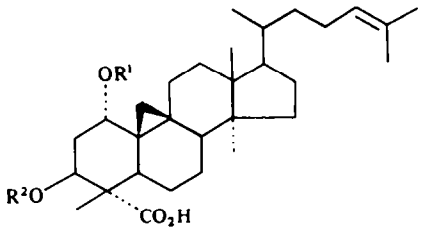
In continuation of our work on the acetone extract from *Combretum molle* leaves [1], we recently reported the characterization of the 1α-hydroxycycloartenoid mollic acid (**1**) and its 3β-D-xyloside (**2**) [2]. We now wish to report the identification by comparative NMR studies of mollic acid α-L-arabinoside (**3**) as the minor constituent in the acid fraction from this extract.

## RESULTS AND DISCUSSION

A slight difference in solubility between the glucoside (**4**) and the xyloside (**2**) of mollic acid (**1**) in ethanol enabled compound **4**, the least soluble, to be obtained pure by repeated recrystallization of the acid fraction from the acetone extract [2]. Preparative HPLC separation of the resultant mother liquors yielded xyloside **2** plus a fraction comprised of a mixture of this compound and a minor constituent of similar polarity. This HPLC separation was achieved by shaving fractions from the leading peak which contained the slightly less polar xyloside **2** [2]. Attempts at separating the mixed fraction **5** have thus for not been successful since band tailing of the xyloside **2** results in this compound coeluting with the minor constituent. It was evident from their response to TLC spray reagents in addition to their similarities in polarity that the two compounds were closely related.

This was confirmed by $^1$H NMR and in particular $^{13}$C NMR analysis, which showed that the mixture spectrum differed from the spectrum of the pure xyloside **2**

*Author to whom correspondence should be addressed.




**1** $R^1 = R^2 = H$
**2** $R^1 = H$, $R^2$ = xyl
**3** $R^1 = H$, $R^2$ = ara
**4** $R^1 = H$, $R^2$ = glu
**7** $R^1$ = Ac, $R^2$ = xyl $(Ac)_3$

only in the sugar region, i.e. both compounds have mollic acid (**1**) as their aglycone. Peracetylation of the mixture followed by electron impact (EI) mass spectral analysis showed that the mass spectrum of the acetate mixture **6** was identical with that of mollic acid β-D-xyloside tetraacetate (**7**). Since pentopyranoside isomers cannot be distinguished by EI mass spectrometry [3], this indicated that the minor component of the mixture contained a sugar isomeric with xylose.

With a few exceptions naturally occurring pentopyranosides are confined to D-xylo and L-arabinopentopyranosides and therefore the minor component in the mixture was most likely mollic acid α-L-arabinoside (**3**). In order to confirm this, cholesteryl-α-L-arabinoside triacetate (**8**) and β-D-xyloside triacetate (**9**) were prepared and their mass, $^1H$ and $^{13}C$ NMR spectra compared with the corresponding spectra obtained for **6**, the peracetylated product of mixture **5**. As expected the peaks due to the sugar fragments in the mass spectrum of **6**, **8** and **9** are identical (Table 1), while in the corresponding $^1H$ NMR spectra signals due to protons in the sugar moieties of **8** and **9** could be identified in the spectrum of **6** in spite of interference by the aglycone H-1 acetoxymethine and H-24 olefinic proton signals. Unequivocal proof that α-L-arabinoside was the sugar in the minor component was furnished by comparing the sugar region of the $^{13}C$ NMR spectra of **8** and **9** and their deacetylated equivalents **10** and **11** with the spectra given by **5** and **6**. This comparison is illustrated in Fig. 1. Apart from expected variations in the positions of the C-1′ and C-2′ signals due to the different ring A environment in the mollic compounds, it is clearly evident that the mixture consists of mollic β-D-xyloside (**2**) and α-L-arabinoside (**3**) in an approximately 2:1 ratio.

## EXPERIMENTAL

*Extraction and isolation of the acid fraction.* This was carried out as described previously [1, 2].

*Isolation of the mixed fraction* **5**. HPLC separations of fractions enriched in mollic acid xyloside (**2**) on a Waters Associates Prep LC/System 500 (A) fitted with a 1 inch semi-preparative column (P/N 84980) packed with Merck silica gel (7731) using a solvent gradient of EtOAc–EtOH (1–10%) yielded pure **2** (450 mg), and the mixed fraction **5** (120 mg). $^{13}C$ NMR (20 MHz, $C_5D_5N$, sugar region only): see Fig. 1; δ106.6 (xyl, C-1′), 105.7 (ara, C-1′) 81.5, (C-3); 78.2 (xyl, C-3′), 75.6 (xyl, C-2′), 74.4 (ara, C-3′), 73.0 (ara, C-2′), 72.5 (C-1), 71.2 (xyl, C-4′), 69.4 (ara, C-4′) 67.1 (xyl, C-5′), 66.5 (ara, C-5′).

*Acetylation of mixture* **5**. Acetylation of **5** (100 mg) following usual methods gave the peracetylated mixture **6** (90 mg) as colourless needles (MeCN–EtOH). $^{13}C$ NMR (20 MHz, $CDCl_3$, sugar region only, see Fig. 1): δ101.6 (ara, C-1′), 101.4 (xylo, C-1′), 80.1 (C-3), 75.3 (C-1), 71.3 (xylo, C-2′), 70.5 (xylo, C-3′), 69.9 (ara, C-3′) 68.8 (ara, C-2′; xylo, C-4′), 67.4 (ara, C-4′), 62.7 (ara, C-5′), 61.8 (xylo, C-5′); EIMS (70 eV, 200°; sugar region only): see Table 1.

*Glycosylation reactions.* Cholesteryl-α-L-arabinopyranoside triacetate (**8**) and cholesteryl-β-D-xyloside triacetate (**9**) were prepared from cholesterol (2 g) by a modified Koenigs–Knorr procedure [4] using the appropriate 2,3,4-tri-*O*-acetyl pyranosyl bromide (2 mole excess). The required products were separated from their anomers using the HPLC procedure described above and a solvent gradient consisting of petrol–EtOAc (1–50%). Except for the sugar region, the NMR spectra of these two compounds were the same as those given by cholesterol.

Cholesteryl-α-L-arabinoside triacetate (**8**, 389 mg), mp 168–169° (MeCN); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1730, 1450, 1355, 1255, 1245, 1210, 1135, 1090, 1040, 995; $^1H$ NMR (90 MHz, $CDCl_3$, sugar region only): δ5.27 (*d*, H-6), 5.18–5.03 (H-2′, H-3′, H-4′) 4.46 (*d*, $J = 6$ Hz, H-1′), 4.07–3.48 (2 × H-5′); $^{13}C$ NMR (20 MHz, $CDCl_3$, sugar region only; see Fig. 1): δ99.3 (C-1′), 78.9 (C-3), 69.9 (C-3′) 69.1 (C-2′), 67.5 (C-4′), 62.8 (C-5′); EIMS (70 eV, 200°; sugar region only): see Table 1.

Cholesteryl-β-D-xyloside triacetate (**9**, 643 mg), mp 160–162° (MeCN); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1730, 1450, 1420, 1350, 1235, 1210, 1125, 1050, 1030, 1015, 995; $^1H$ NMR (90 MHz, $CDCl_3$; sugar region only): δ5.27 (*d*, H-6), 5.12–4.71 (H-2′, H-3′, H-4′), 4.50 (*d*, $J = 7$ Hz, H-1′), 4.14–3.80 (2 × H-5′); $^{13}C$ NMR (20 MHz, sugar region only): δ99.3 (C-1′), 79.0 (C-3), 71.6 (C-2′) 71.0 (C-3′), 68.8 (C-4′), 61.9 (C-5′); EIMS (70 eV, 200°; sugar region only): see Table 1.

*Hydrolysis of* **8** *and* **9**. Each of these compounds (150 mg) dissolved in a minimum of $CHCl_3$ was stirred with an ethanolic soln of NaOEt until TLC showed the hydrolysis to be complete.

Table 1. Mass spectra of compounds **6**–**9** giving ions due to the sugar fragments

| Compounds | *m/z* (rel. int.) |
|---|---|
| Cholesteryl-α-L-arabinoside triacetate (**8**) | 259 (17), 199 (14), 157 (43), 139 (79), 97 (66), 43 (100) |
| Cholesteryl-β-D-xyloside triacetate (**9**) | 259 (16), 199 (35), 157 (62), 139 (78), 97 (68), 43 (100) |
| Mollic acid glycoside tetraacetate mixture (**6**) | 259 (17), 199 (19), 157 (25), 139 (41), 97 (34), 43 (100) |
| Mollic acid β-D-xyloside tetraacetate (**7**) | 259 (42), 199 (48), 157 (10), 139 (70), 97 (90), 43 (100) |

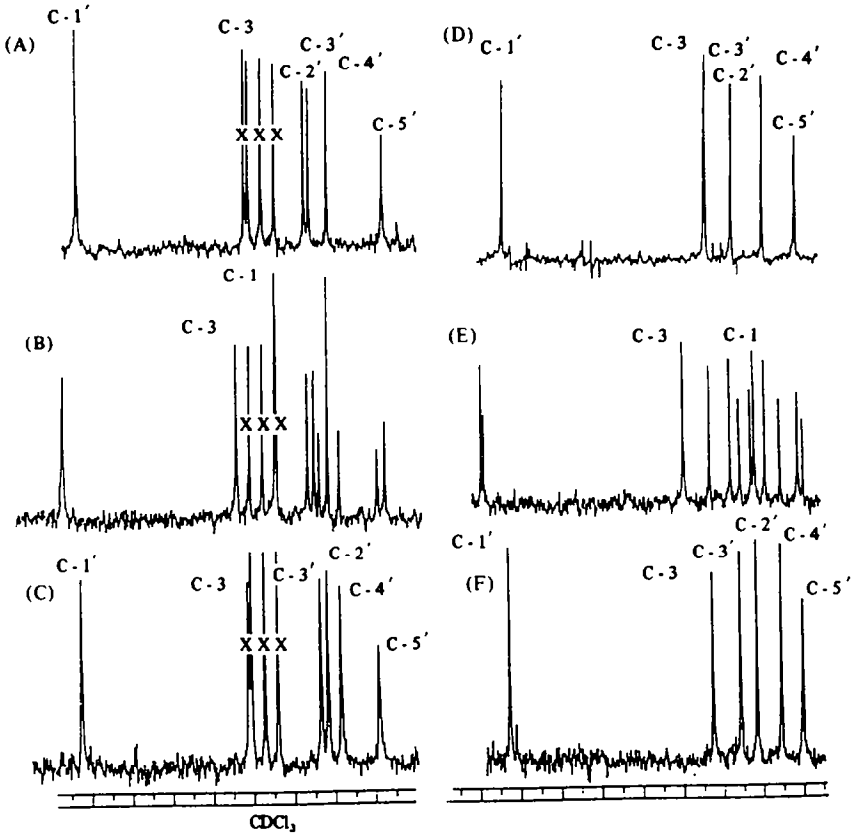



Fig. 1 $^{13}C$ NMR spectra of compounds **5**, **6** and **8**–**11** showing the sugar region. (A) Cholesteryl-β-L-xyloside triacetate (**9**); (B) mollic acid glycoside tetraacetate mixture (**6**); (C) cholesteryl-α-L-arabinoside triacetate (**8**); (D) cholesteryl-β-D-xyloside (**11**); (E) mollic acid glycoside mixture (**5**); (F) cholesteryl-α-L-arabinoside (**10**). Compounds **6**, **8** and **9** were measured in $CDCl_3$, compounds **9**, **10** and **11** in $C_5D_5N$.

The resultant precipitates were collected and crystallized from EtOH to give **10** and **11**.

Cholesteryl-α-L-arabinoside (**10**, 100 mg), mp 210–215° (dec.; EtOH); $^{13}C$ NMR (20 MHz, $C_5D_5N$, sugar region only, see Fig. 1): δ103.3 (C-1'), 78.2 (C-3), 74.8 (C-3'), 72.7 (C-2'), 69.7 (C-4'), 67.0 (C-5').

Cholesteryl-β-D-xyloside (**11**, 98 mg), mp 238–245° (dec.; EtOH); $^{13}C$ NMR (20 MHz, $C_5D_5N$, sugar region only, see Fig. 1): δ103.6 (C-1'), 78.7 (C-3), 78.5 (C-3'), 75.2 (C-2'), 71.4 (C-4'), 67.3 (C-5').

*Acknowledgements*—We thank Dr D. H. Picnaar for running the NMR spectra, the University for financial assistance, and Professor K. H. Pegel for his support and helpful suggestions.

## REFERENCES

1. Pegel, K. H. and Rogers, C. B. (1975) *Tetrahedron Letters* 4299.
2. Pegel, K. H. and Rogers, C. B. (1985) *J. Chem. Soc. Perkin Trans.* 1, 1711.
3. Biemann, K., De Jongh, D. C. and Schnoes, H. K. (1963) *J. Am. Chem. Soc.* **85**, 1763.
4. Papaphilippou, A. and Pegel, K. H. (1979) *S. Afr. J. Chem.* **32**, 187.