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STRUCTURE PROOF OF MURICATIN B: 11-HYDROXY HEXADECANOIC ACID DIRHAMNOSIDE*

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Key Word Index—*Ipomoea muricata*; Convolvulaceae; muricatin B; structure proof by synthesis; glycosidic acid; ^{13}C-NMR.

Abstract—Synthesis of (+)-11-hydroxyhexadecanoic acid-*O*-(4-*O*-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside showed that muricatin B from *Ipomoea muricata* does not contain the proposed β- but an α-linked dirhamnoside moiety. This is the first reported synthesis of a naturally occurring *O*-glycoside of a long chain hydroxy-fatty acid.

In the course of our work on the structure of glycoresins with laxative action in different members of the Convolvulaceae, we have elucidated the structure of several glycosidic acids [1–3]. They are branched oligosides of long chain hydroxy fatty acids containing four, five or six hexoses including deoxyhexoses. Proof of structure by synthesis for such complex compounds is at present not within reach. This is due to the lack of efficient methods for assembling the oligoside moieties. Muricatin A and B from *Ipomoea muricata* are two rare naturally occurring glycosidic acids with a disaccharide moiety [4, 5]. For muricatin A and B the structures of a 14-*O*-diglucoside of ethyl-4,14-dihydroxystearate and 4-*O*-α-L-rhamnopyranosyl-α-L-rhamnopyranoside of 11-hydroxyhexadecanoic acid respectively have been postulated by Khanna and Gupta [5]. The fact that the authors postulated the very unusual β-linkage between the two rhamnose units in muricatin B prompted us to synthesize this compound to confirm or disprove the structure. Since the negative optical rotation of muricatin B in accordance with the Klyne rule [6] was consistent only with an α-linkage between the two sugars, we synthesized the hexaacetate of 4-*O*-α-L-rhamnopyranosyl-α-L-rhamnopyranose. Benzyl 2,3-isopropylidene-α-L-rhamnopyranoside (**1**) [7] served as the starting material and was condensed with 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (**2**) [8] in benzene–nitromethane in presence of $Hg(CN)_2$. We obtained a coupled product which after purification on a Si gel column was identified as the expected benzyl 2,3-*O*-isopropylidene-4-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**3**). In the ^{1}H-NMR spectrum of **3**, in presence of $Eu(fod)_3$, the anomeric C-1 proton appeared as a doublet at δ 6.61 ppm. Deacetylation of **3** by the method of Zemplen [9] yielded benzyl 2,3-*O*-isopropylidene-4-*O*-(α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**4**) and subsequent acid hydrolysis under mild conditions and acetylation gave benzyl 4-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-2,3-di-*O*-acetyl-α-L-rhamnopyranoside (**5**). Catalytic debenzylation over Pd/C and subsequent acetylation resulted in the hexaacetate of the disaccharide (**6**). The ^{1}H-NMR spectrum showed the H-1 signal at $\delta = 6.26$ ppm as a doublet, $J = 2$ Hz, and the interglycosidic proton also as a doublet with $J = 2$ Hz centred at $\delta = 5.02$ ppm‡.

Treatment of the disaccharide peracetate with HBr in dichloromethane resulted in the α-glycosyl bromide (**7**). Condensation of **7** with (+)-11-hydroxyhexadecanoic acid methyl ester (**8**) in benzene–nitromethane in the presence of $Hg(CN)_2$ was carried out according to the method recently devised for the synthesis of hydroxy fatty acid-*O*-glycosides [11]. The resulting glycosidic acid methyl ester pentaacetate (**9**) had an $[\alpha]_D$ value of −42.4° ($CHCl_3$). Deacetylation of (**9**) by the method of Zemplen followed by treatment of the product with aqueous alkali gave the free glycosidic acid (**10**), the optical rotation of which was nearly the same as that of the natural product§. The ^{13}C-NMR spectrum of **10** was in full agreement with the structure having two α-linked L-rhamnoside moieties. Lack of authentic muricatin B precluded a direct comparison.

EXPERIMENTAL

Mps are uncorr. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian A 60-A and Varian XL-100 NMR spectrometers respectively. MS were taken with an AEI MS 30 instrument. TLC was carried out on Si gel (Merck) with the solvents A: toluene–EtOAc (1:1) and B: EtOAc–MeOH–H_2O (200:33:27); detection: 10% H_2SO_4 and heating to 120°.

Benzyl 2,3-O-isopropylidene-α-L-rhamnopyranoside (**1**). Benzyl α-L-rhamnopyranoside (11 g) was dissolved in dry Me_2CO (550 ml) containing 1.65 ml conc H_2SO_4. After shaking for 5 hr the soln was neutralized with conc NH_4OH, filtered and evapd. From the residue, which was dissolved in hot hexane, a crystalline product was obtained (9.43 g, 76.8%) mp 73–75°, $[\alpha]_D^{26}$ −59.5° ($c = 0.8478$, in $CHCl_3$) (lit. [7] mp 73–75°, $[\alpha]_D^{20}$ −55°) *Rf* in solv. **B** = 0.92. $C_{16}H_{22}O_5$ (294.3) Calcd. C 65.29 H 7.54 Found C 65.18 H 7.0.

Benzyl 2,3-O-isopropylidene-4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**3**). 2.34 g (0.01 ml) of **1** and dry $Hg(CN)_2$ (0.1 g) were dissolved in 250 ml C_6H_6–nitromethane (1:1) and concd to 80 ml. To this soln 4.27 g (0.012) of α-acetobromorhamnose (**2**) [8] were added under exclusion of moisture. After stirring for 24 hr at 40° the soln was evapd and the resulting syrup boiled with 100 ml CH_2Cl_2, filtered, and the filtrate was successively washed twice with 2% aq.

* Part 4 in the series 'Chemical components of the Convolvulaceae resins': for Part 3, see ref. [1].

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‡ After completion of our work we came across a report [10] by Bebault *et al.* on the synthesis of methyl 4-*O*-α-L-rhamnopyranosyl-α-L-rhamnopyranoside using an analogous method. Instead of **1** as the starting material, the authors used the methyl glycoside analogue.

§ The small difference in the rotation could be ascribed to the fact that our synthetic product could not be crystallized.

NaI and thrice with ice H_2O. After drying, the solv. was concd to a syrup and the major component (TLC solv. A R_f 0.79) separated by column chromatography on Si gel using solvent A. The yield of **3** was 4.25 g (75.3%) mp 109–110°, $[\alpha]_D^{25}$ −81.6° (c = 0.9787 in $CHCl_3$). $C_{28}H_{38}O_{12}$ (566.6) Found: C 59.77, H 6.27 Calcd. C 59.35, H 6.76. NMR ($CDCl_3$) δ = 1.1–1.6 ppm (12 H, C-Me protons), 1.9–2.2 (9 H, $CH_3 \cdot CO$—), 3.35–5.35 (12 H, —CH_2—Ph and sugar protons), 7.4 (5 H, phenyl protons).

Benzyl2,3-O-isopropylidene-4-O-α-L-rhamnopyranosyl-α-L-rhamnopyranoside (**4**). 4 g of **3** were saponified according to ref. [9] in abs. MeOH with NaOMe. After neutralization and concn to a syrup, **4** could be obtained from Me_2CO in crystalline form 2 g, 64.2%) mp 78–800°; $[\alpha]_D^{23}$ −99.6° (c = 0.92 in Me_2CO). R_f 0.83 in solv. B, $C_{22}H_{32}O_9$ (440.5) Calcd. C 59.98, H 7.32 Found C 59.29, H 7.24. NMR ($CDCl_3$) δ = 1.15–1.48 ppm (12 H, C-Me protons), 3.3–4.25 (8 H, sugar protons), 4.7 (*q*, 2 H, O—CH_2—Ph), 5.05 (*s*, 1 H, H-1'), 5.2 (1 H, *d*, J = 2 Hz, H-1), 7.35 (*s*, 5 H, phenyl protons).

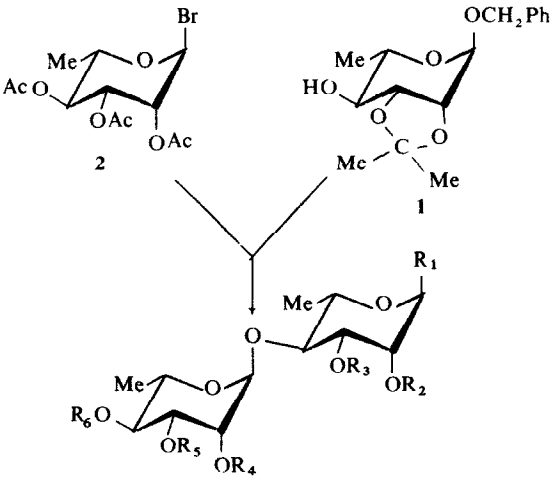
Benzyl 2,3-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**5**). 2 g of **4** were dissolved in 20 ml 50% HOAc and heated for 2 hr at 80°. After adding toluene and EtOH, the HOAc was removed by distillation and the resulting syrup (1.6 g, 88.3%, R_f in solv. B = 0.58, immediately acetylated with Ac_2O–Py (2:1) *ca* 18 hr at room temp. The mixture was poured into the 10 fold amount of ice H_2O and after 4 hr the supernatant decanted. The resulting syrup was dissolved in $CHCl_3$, washed with 2% $NaHCO_3$ soln and ice H_2O, and the $CHCl_3$-phase brought to dryness. Crystallization from EtOH-H_2O yielded 2 g (76%) of **5** mp 94–102° $[\alpha]_D^{25}$ −67.6 (c = 0.8748 in $CHCl_3$); R_f in solv. B = 0.67; $C_{29}H_{38}O_{14}$ (610.59) Found C 56.43, H 6.04; Calcd. C 57.04, H 6.27. NMR ($CDCl_3$): δ 1.2 ppm (3 H, *d*, J = 6 Hz Me-rhamnose); 1.35 (3 H, *d*, J = 6 Hz Me-rhamnose); 1.95–2.1 (15 H, MeCO—); 3.4–5.3 (12 H, sugar protons, O—CH_2—Ph); 7.3 (5 H, phenyl protons).

4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-1,2,3-di-O-acetyl-α-L-rhamnopyranose (**6**). 2 g of **5** were dissolved in 20 ml EtOAc, which contained 5% HOAc and hydrogenated at 2 atm. *ca* 18 hr in the presence of Pd/C. After filtration and concn of the solution to a syrup (1.5 g, 88%, R_f in solv. A = 0.50), acetylation was carried out with Ac_2O–Py in the usual manner. Crystallization from 50% EtOH. Mp 150–152° (lit. mp [10] 162–163°), $[\alpha]_D^{25}$ −60.9 (c = 0.8535 in $CHCl_3$, (lit. [10] $[\alpha]_D$ −63.6°); R_f in solv. A = 0.67. $C_{24}H_{34}O_{15}$ (562.5); Found: C 51.40, H 6.12 Calcd. C 51.24, H 6.09. NMR ($CDCl_3$): δ = 1.2 ppm (3 H, *d*, J = 6 Hz, Me-rhamnose); 1.35 (3 H, *d*, J = 6.0 Hz, Me-rhamnose), 2.0–2.2 (18 H, MeCO—); 3.6–5.5 (9 H, sugar protons); 5.8 (1 H, *d*, J = 2 Hz, H-1).

2,3-Di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl bromide (**7**). 1.6 g of **6** were dissolved in 2 ml CH_2Cl_2 and cooled to 0°. HBr in HOAc (5 ml, 40%) was then added dropwise, the mixture kept at 0° for 3 hr and worked up as usual to yield 0.69 g (34.7%) of the crystalline bromide mp 191–193°. R_f 0.68 solv. A. $C_{22}H_{31}O_{13}$ Br (583.4) Calcd. C 45.29, H 5.36; Found C 45.33, H 5.41. NMR ($CDCl_3$) δ = 1.3 ppm (*d*, 3 H, J = 12 Hz, rhamnose-Me), 1.38 (*d*, 3 H, J = 12 Hz, rhamnose-Me), 2.0–2.15 (15 H, MeCo) 3.5–5.8 (9 H, sugar protons), 6.3 (*d*, 1 H, J = 2 Hz, H-1).

Muricatin B-pentaacetate methyl ester (**9**). Methyl-11-hydroxyhexadecanoate (**8**) (0.66 g), isolated from the root of *Convolvulus scammonia* [12] and 0.288 g of $Hg(CN^2)$ were dissolved in 50 ml nitromethane-C_6H_6 (1:1) mixture and concd to 30 ml. The bromide **7** (0.66 g) was added and the mixture stirred for 3 hr at 40°. After evapn of the solvent the residue was boiled with $CHCl_3$ (50 ml), filtered and the filtrate washed twice with a 2% NaI soln followed by H_2O. The $CHCl_3$ soln was dried, evapd to a syrup and then subjected to column chromatography over Si gel. Elution with hexane–Et_2O (1:1) removed the unreacted hydroxy fatty acid ester while the Et_2O–hexane (3:2) fraction contained the condensation product (0.31 g, 35%) $[\alpha]_D^{24}$ −41.7° (c = 0.9217 in $CHCl_3$), R_f 0.76 solv. A; $C_{39}H_{64}O_{16}$ (788.9) Calcd. C 59.38, H 8.17; Found 58.62, H 7.94. The substance could not be recrystallized. NMR ($CDCl_3$): δ = 0.95 ppm (*t*, 3 H, J = 5 Hz, ω-Me), 1.1–1.85 (24 H), 1.9–2.25 (18 H, Me CO), 2.35 (*t*, 2H, J = 7 Hz, CH_2 − CO), 3.5–5.5 (14H, sugar protons, MeO—, CH—O-11).

Muricatin B (**10**). 0.3 g of **9** was dissolved in abs. MeOH and treated with 0.1 ml of 0.5 N NaOMe soln. The resulting muricatin methyl ester was subsequently saponified by refluxing with 0.2 N NaOH soln for 5 hr. The soln was neutralized with HOAc and evapd to a syrup and purified by column chromatography on Si gel using solv. B. Muricatin B was thus obtained as a syrupy substance (180 mg) which could not be crystallized. $[\alpha]_D^{26}$ −56.6° (c = 0.4948 in EtOH) lit. [5], $[\alpha]_D$ −46°. R_f 0.66 in solv. B. ^{13}C-NMR ($CDCl_3$) 174.7 ppm (C-1), 101.5 (C-1'), 99.1 (C-1''), 77.0 (C-11), 72.4 (C-4''), 71.8 (C-2', C-3'), 71.2 (C-2''), 70.9 (C-3''), 69.1 (C-5''), 67.2 (C-5'), 34.4, 34.0, 33.2 (3 carbons), 29.4 to 28.9 (5 carbons), 24.7 (3 carbons), 22.22 and 13.9 (2 carbons of hydroxy fatty acid), 18.1, 17.8 (C-6', C-6'').



	R_1	R_2	R_3	R_5	R_6
3	OCH_2Ph	Me	Me	Ac	Ac Ac
4	OCH_2Ph	Me	Me	H	H H
5	OCH_2Ph	Ac	Ac	Ac	Ac Ac
6	OAc	Ac	Ac	Ac	Ac Ac
7	Br	Ac	Ac	Ac	Ac Ac

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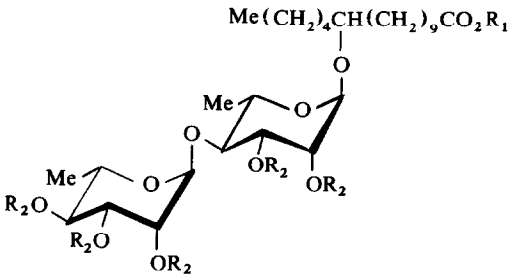
7 $+Me(CH_2)_4CHOH(CH_2)_9CO_2Me \rightarrow$

8



	R_1	R_2
9	Me	Ac
10	H	H

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EPICUTICULAR WAXES OF TWO SORGHUM VARIETIES

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Key Word Index—*Sorghum vulgare*; Gramineae; sorghum; epicuticular wax.

Abstract—The epicuticular waxes of the two sorghum varieties Alliance A and SD 102 have been analyzed, after separation of the leaf blades from the sheaths. The major constituents were found to be free fatty acids but small amounts of esters, aldehydes, alcohols, *n*-alkanes and sterols were also detected. The typical chain lengths of aldehydes, free alcohols and free fatty acids were C_{28} and C_{30}.

INTRODUCTION

In a previous communication [1] we reported preliminary results on the composition of the leaf waxes of sorghum varieties, Alliance A and SD 102. The investigation has now been completed, analyzing separately the waxes from the leaf blades and the leaf sheaths.

RESULTS AND DISCUSSION

In sorghum, wax is concentrated mainly on the leaf sheath and the lower part of the leaf blade. Surface wax, a white soft material, wears off readily by the brushing of the leaf sheaths against neighbouring plants so that on older leaves the sheaths appear less wax covered and at the tasseling stage the most glaucous part of the plant is leaf sheath. As a practical and reliable test for detecting the wax on the different part of the plant, we made use of the property that waxy leaf surfaces reflect light strongly when dipped in water [2].

As can be seen in Table 1, there was a marked similarity in the amount of wax per plant of the two sorghum lines and, in contrast to what was apparent to the naked eye, the blades yielded a larger amount of wax than the sheaths, although the surface area of the two parts were not taken into account. A comparison was also made of the composition of the wax obtained from the blades and the sheaths of the two varieties. Free fatty acids were found as the major wax components of the leaf sheaths of both varieties and of the blades of SD 102 sorghum (Table 1). However, in the case of Alliance A leaf blades, esters constituted the major constituents, comprising 44.2% of the wax and fatty acids only 23.3%. Amounts of *n*-alkanes and free alcohols were relatively small in all wax fractions. The data in Table 1 also indicate that there is an inverse relationship between the amounts of esters and free fatty acids; thus the percentage of the latter class of compounds is low when that of esters is high.

Table 2 shows the chain length distribution in the wax components. *n*-Alkanes have the usual composition with major C_{27}, C_{29} and C_{31} homologues. The acids and alcohols obtained by acid methanolysis of esters, have a wide chain length range without any long chain major components. The alcohol composition is unusual in this respect since wax esters from other normal cereal lines generally give alcohols with major homologue, usually C_{26} or C_{28} for Gramineae [3, 4], C_{32} for maize [5]. The mass spectra of the esters showed that the major homologues were in the range C_{40}–C_{50}.

The free alcohols of sorghum are also unusual in that there are two major components, the dominant C_{28} and substantial amounts of C_{30}. The alcoholic fraction of the wax from the leaf sheaths of SD 102 contains in addition to the alcohols 37.5% of sterols. The sterol fraction was comprised of at least three components, each characterized by the three ions 426 M^+, 411 $(M\text{-}Me)^+$ and 393 $(M\text{-}Me\text{—}H_2O)^+$. These spectra are similar to those obtained for various plant sterols by other workers