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PARTIALLY-ACETYLATED DODECANYL TRI- AND TETRA-RHAMNOSIDE DERIVATIVES FROM Cleistopholis glauca (ANNONACEAE)

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Summary: Five partially-acetylated derivatives (1)-(5) of 1-O-dodecanyl α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside have been isolated from the stem bark of Cleistopholis glauca (Annonaceae) and their structures elucidated by using COSY, delayed COSY, and FAB Mass spectroscopy. The trisaccharide 1-O-dodecanyl α-L-2,3,4-triacetylrhamnopyranosyl-(1→3)-α-L-4-acetylrhamno-pyranosyl-(1→4)-α-L-rhamnopyranoside (6) was also isolated.

The isolation of several biologically active acetogenin derivatives from the Annonaceae[1] encouraged us to re-examine the stem bark of the Cameroon species Cleistopholis glauca. Previous work reported only sesquiterpenoids.[2] Extraction of the stem bark with chloroform followed by chromatography afforded six new compounds (1)-(6). It was clear from the ^{1}H and ^{13}C n.m.r. spectra of these compounds that they were partially-acetylated oligosaccharides containing an n-alkyl ether. The structural elucidation was simplified by the fact that compounds (1)-(5) afforded the same peracetate (7) m.p. 140-143°C.

Analysis of the 360 MHz ^{1}H n.m.r. spectrum of (7) revealed four sets of protons attributable to rhamnose residues (see Table I). These protons were readily assigned to their respective rings by a COSY experiment. Inspection of the chemical shifts indicated that the rings are joined by two (1→3) and one (1→4) links. The protons of the oxygenated methylene group [δ_C 68.2 (t)] of the alkyl chain resonate at δ_H 3.64 (dt, J 10.2, 6.8 Hz) and δ_H 3.40 (dt, J 9.6, 6.8 Hz). The dodecanyl nature of the alkyl chain was determined by acidic hydrolysis of (2) which fortuitously afforded 1-O-dodecanyl α-rhamnopyranoside (8) whose FAB mass spectrum showed $(M + Na)^+$ at m/z 355 and fragment ions corresponding to $C_{12}H_{25}O$ (m/z 185) and $C_6H_{11}O_4$ (m/z 147). This was confirmed in the FAB mass spectrum of the peracetate (7) itself which showed $(M + H)^+$ at m/z 1149 and fragment ions at m/z 963, 733, 503, and 273 corresponding to sequential cleavage of the acetal bonds. In most compounds

of the series a small peak attributable to a bis-homologue (tetradecanyl) is observed.

The order of the sugar residues in the peracetate (7) was readily solved using delayed COSY (at 300 MHz) with very long delays (e.g. 250 ms) before and after the mixing pulse. This method has recently been used effectively in the structural elucidation of triterpenoid saponins from alfalfa.[3] The correlations of 1a-H, 1b-H, and 1c-H shown in figure 1 clearly reveal the glycosidic links 1a→4, 1b→3a, and 1c→3b. Correlations of 1-H with the methylene protons of the dodecanyl chain were not observed under the conditions of the experiment. The above results lead unambiguously to structure (7) for the peracetate with the exception of the anomeric configurations. The coupling of 1-H of α- and β-rhamnose derivatives is not a reliable indication of configuration. In the mixture of anomers obtained on acetylation of rhamnose 1-H of the α-isomer appears at δ_H 5.92 as a doublet of doublets (J 1.9, 0.6 Hz) while 1-H of the β-isomer is a doublet (J 1.2 Hz) at δ_H 5.76. It was apparent that NOE difference experiments on (7) should yield definitive answers, as for rhamnose peracetate (at 200 MHz) irradiation of 1-H of the α-anomer gave a substantial NOE only at 2-H while irradiation of 1-H of the β-anomer resulted in substantial NOEs at 2-H, 3-H, and 5-H. In the case of (7) (at 200 MHz) irradiation of 1-H (δ_H 4.66) gave NOEs at 2-H (δ_H 5.21, 3%) and at one of the resonances (δ_H 3.38, 3%) of the terminal methylene group of the dodecanyl unit. Irradiation of 1a-H (δ_H 4.94) afforded NOEs at 2a-H (δ_H 5.01, 5%) and also at 4-H (δ_H 3.64, 5%). Similarly irradiation of the remaining resonances 1b-H (δ_H 4.85) and 1c-H (δ_H 4.84) yielded NOEs at the corresponding 2b-H (δ_H 4.79, 3%) and 2c-H (δ_H 5.05, 4%) and also at 3a-H (δ_H 3.98, 5%) and 3b-H (δ_H 3.92, 5%) respectively. These NOE results clearly establish the presence of α-rhamnose units as in (7) and also provide confirmation of the glycosidic links determined above by delayed COSY.

The structures of the natural tetrarhamnoside derivatives[4] were elucidated using the same combination of COSY and delayed COSY. This approach was effective for the penta-acetate (5), the tetra-acetates (4) and (3), and the triacetate (2). The most polar compound, the diacetate (1), gave broad spectra in $CDCl_3$. In CD_3OD at 34°C the spectra were much sharper and the appropriate correlations were observed. The absolute configuration of the rhamnose was established by hydrolysis of the triacetate (2) in TFA. This yielded L(+)-rhamnose {$[\alpha]_D$ +11.5 (c, 0.54 in MeOH)}.

The spectroscopic properties of the remaining compound, (6) m.p. 64-67°C indicated that it is a trirhamnose derivative containing four acetates and a dodecanyl ether [$(M + Na)^+$ 815]. The FAB mass spectrum suggests that three acetates are located on the terminal ring (m/z 273) and one is on the central ring (m/z 461). The acetylated positions are easily identified by their ^1H n.m.r. chemical shifts as 1 x 2-C, 1 x 3-C, and 2 x 4-C. Acetylation

afforded a peracetate which has "additional" acetates at 2 x 2-C and 1 x 3-C. The glycosidic links must therefore involve 1-C→4-C and 1-C→3-C. A normal COSY experiment confirmed the presence of 2,3,4-triacetylated and 4-monoacetylated rhamnose residues. These results, in conjunction with the FAB data above, lead to the structure (6), 1-O-dodecanyl α-L-2,3,4-triacetylrhamnopyranosyl-(1→3)-α-L-4-acetylrhamno-pyranosyl-(1→4)-α-L-rhamnopyranoside, for the trisaccharide.

Table 1. ^{1}H n.m.r. Chemical Shifts of Peracetate (7) (300 MHz, $CDCl_3$)

	1-H (d)	2-H (dd)	3-H (dd)
	4.66 (1.4)	5.21 (3.4, 1.6)	5.24 (9.4, 3.4)
a	4.94 (1.9)	5.01 (3.1, 2.0)	3.98 (9.8, 3.2)
b	4.85 (1.7)	4.97 (3.4, 1.7)	3.92 (9.9, 3.4)
c	4.84 (1.8)	5.05 (3.5, 1.9)	5.15 (10.2, 3.3)

	4-H (t)	5-H (dq)	6-H_3 (d)
	3.64 (9.3)	3.80 (9.3, 6.2)	1.34 (6.2)
a	5.07 (9.8)	3.89 (9.8, 6.3)	1.22 (6.4)
b	5.06 (9.7)	3.71 (9.7, 6.3)	1.16 (6.3)
c	5.02 (10.0)	3.81 (9.6, 6.3)	1.17 (6.3)

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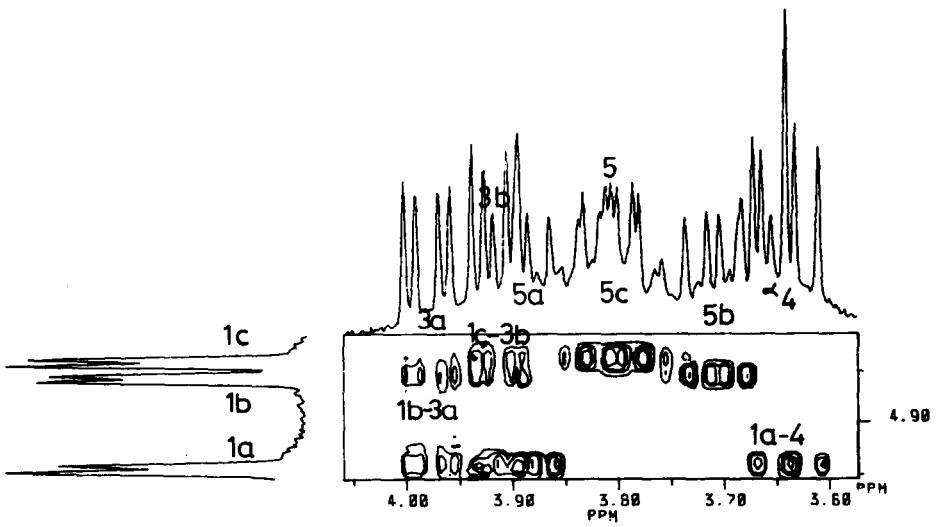
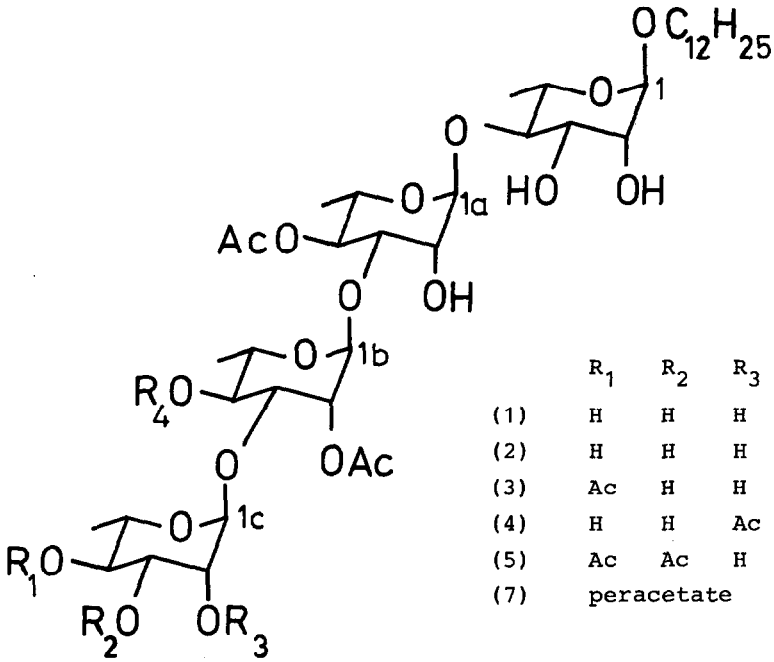
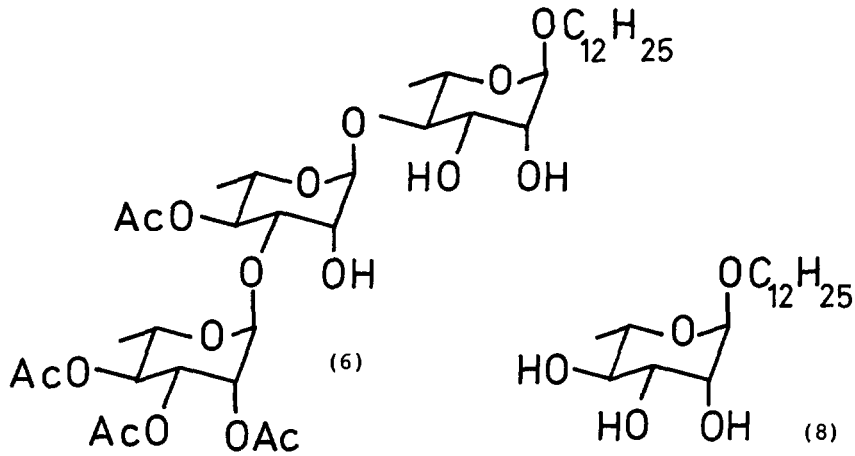


Figure 1. Delayed COSY experiment on the peracetate (7).



	R_1	R_2	R_3	R_4
(1)	H	H	H	H
(2)	H	H	H	Ac
(3)	Ac	H	H	Ac
(4)	H	H	Ac	Ac
(5)	Ac	Ac	H	Ac
(7)	peracetate			



(6)

(8)

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2. P.G. Waterman, Phytochemistry, 1986, 25, 1.
3. G. Massiot, C. Lavaud, D. Guillaume, L. Le Men-Olivier, and G. Van Binst, J. Chem. Soc., Chem. Commun., 1986, 1485.
4. Diacetate (1) m.p. 117-120°C; triacetate (2) m.p. 107-109°C, m/z 920 $(M + Na)^+$; tetra-acetate (3) m.p. 105-107°C, m/z 961 $(M + Na)^+$; tetra-acetate (4) m.p. 98-100°C, m/z 961 $(M + Na)^+$; penta-acetate (5) m.p. 90-93°C, m/z 1003 $(M + Na)^+$.

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