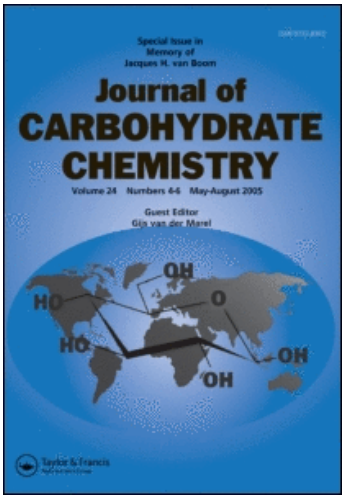

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Tiwari, Kamal N. , Khare, Anakshi and Khare, Maheshwari p.(1984) 'Structure of Vimose', Journal of Carbohydrate Chemistry, 3: 2, 315 — 330
To link to this Article: DOI: 10.1080/07328308408058823
URL: http://dx.doi.org/10.1080/07328308408058823

J. CARBOHYDRATE CHEMISTRY, 3(2), 315-330 (1984)

STRUCTURE OF VIMOSE

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Received August 15, 1983

ABSTRACT

The structure of vimose, a novel tetrasaccharide isolated from the dried twigs of Orthenthera viminea (Family: Asclepiadaceae) has been established as O-β-L-diginopyranosyl-(1→4)-O-β-L-diginopyranosyl-(1→4)-O-β-L-diginopyranosyl-(1→4)-α-L-diginopyranose 1 on the basis of spectral and chemical evidence.

INTRODUCTION

The synthesis and identification of the naturally occurring deoxy sugars provides one of the most active areas of research in carbohydrate chemistry. The primary reason for this activity is the widespread occurrence of these compounds in biologically important molecules. Many of these 2-deoxyoligosaccharides are known to occur in nature in cardenolides as oligoglycosides,[1,2] but the isolation of these oligosaccharides of 2-deoxy sugars has not been earlier reported. In our earlier communication,[3] we

0732-8303/84/0302-0315$3.50/0

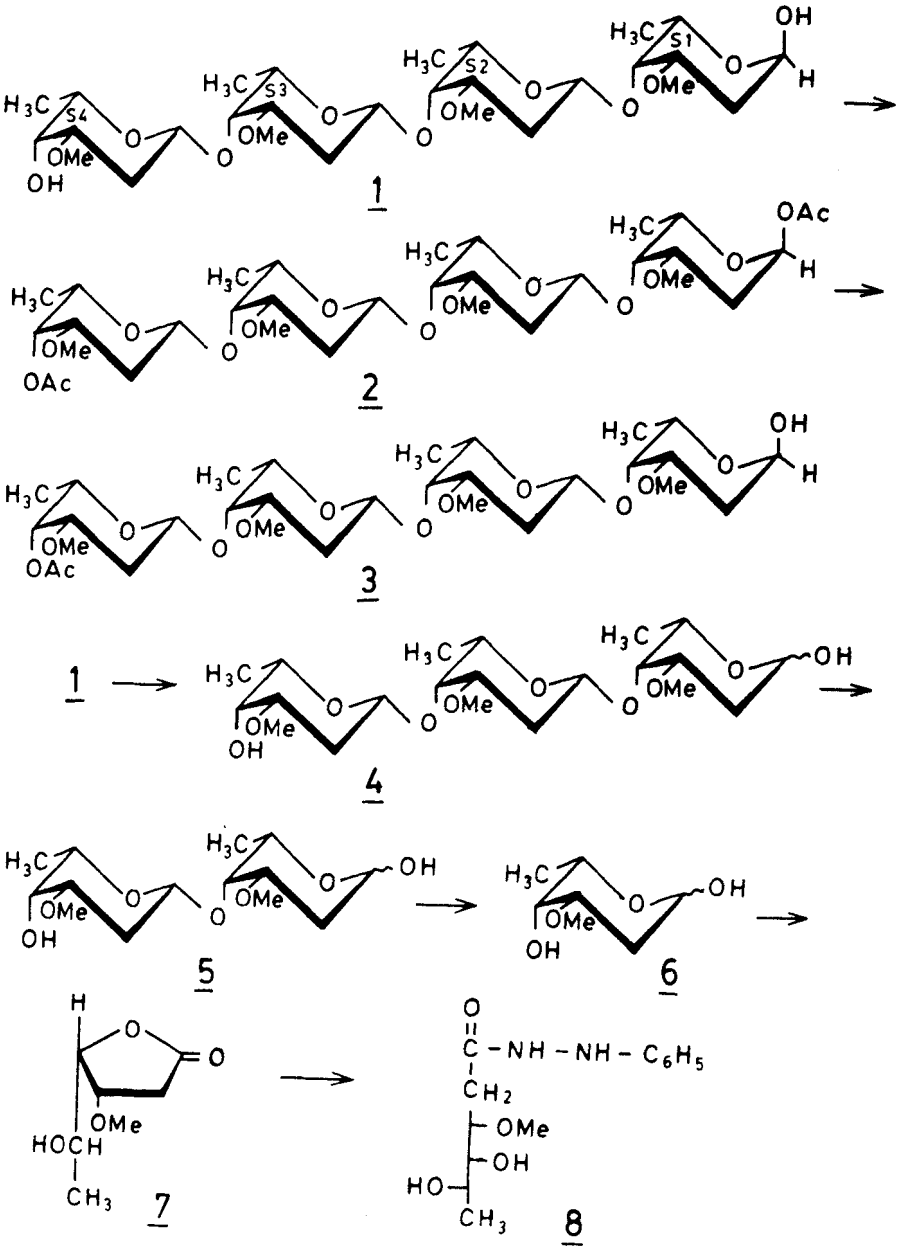
reported the isolation of four novel oligosaccharides from the dried twigs of Orthenthera viminea which were provisionally designated A, B, C, and F in the order of decreasing mobility on paper chromatography (PC). Compounds B and C, named as orthenthose and ornose, have already been characterized as a hitherto unreported class of oligosaccharides of 2,6-dideoxy hexoses viz., L-oleandrotetrose[4] and L-cymarotriose,[5] respectively. The oligosaccharide A, also belonging to this new class, has now been named vimose (1), and its structure is reported in the present communication.

RESULTS AND DISCUSSION

Vimose (1), (amorphous, $[\alpha]_D$ +66°) has an elemental analysis corresponding to that calculated for $C_{28}H_{50}O_{13}$. It reduces Fehling's solution and gives positive tests for 2-deoxy sugars in both the xanthydrol[6] and Keller - Kiliani[7] reactions. In order to identify the sugar units of 1, it was hydrolyzed[8] with 5 mM H_2SO_4 in 1,4-dioxane for 30 min at 50 °C. The hydrolyzate showed the presence of only one product 6, on both TLC and on PC, which was isolated as amorphous substance. A comparison of its rotation and mobility on PC with those of an authentic sample indicated it to be L-diginose[9] (2,6-dideoxy-3-O-methyl L-lyxohexose). For further characterization the sugar 6 from the hydrolyzate was oxidized with bromine water to give an amorphous lactone 7, which had the same

mobility on PC as an authentic sample of L-diginono-1,4-lactone. To obtain a crystalline derivative, the hydrazide 8 was prepared from 7. Vimose (1) thus appeared to be composed of only L-diginose units.

The ^{1}H NMR spectrum of vimose (1) in $CDCl_3$ at 400 MHz not only confirmed that it was a tetrasaccharide of L-diginose, but also ascertained the configuration of the glycosidic linkages. For convenience the four L-diginose units of vimose (1) have been designated as S1, S2, S3 and S4. The spectrum consisted of two singlets of six protons each at δ 3.65 and δ 3.64 attributed to the four methoxyl groups. Two multiplets of two protons each at δ 4.60 - 4.68 and δ 4.20 - 4.32 were assigned to H-5 of the four sugar units. Besides those, there were another two sets of methine proton multiplets of four protons each in the region δ 3.80 - 4.15 and δ 3.20 - 3.56, presumably due to H-3 and H-4, respectively. A one-proton doublet of doublets (J 4 and 1 Hz) centered at δ 5.11, another one-proton doublet of doublets (J 8 and 2 Hz) centered at δ 5.02, and two doublets (J 8 Hz) centered at δ 4.93 and δ 4.83 were assigned to the four anomeric protons in the S1, S4, S2 and S3 units, respectively, of the molecule. The eight H-2 methylene protons of these 2-deoxy sugars appeared as two sets of multiplets of four protons each in the region δ 2.0 - 2.24 and δ 1.65 - 1.80 for the equatorial and axial protons, respectively. In the higher field, one



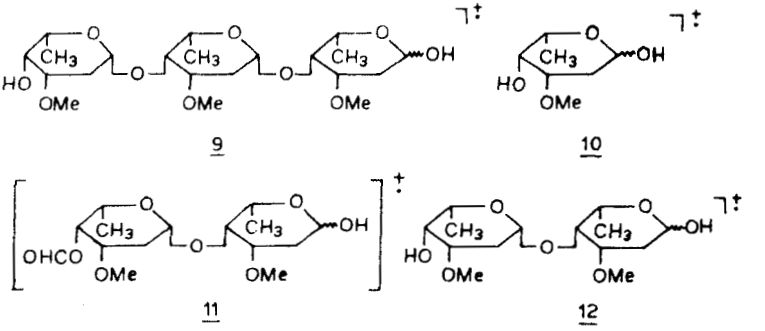
SCHEME 1

doublet of six protons, (J 6 Hz) centered at δ 1.38 and two doublets of three protons each centered at δ 1.31 and δ 1.33 (J 6 Hz) were assigned to the four secondary methyl groups of vimose (1). These assignments were further confirmed by double resonance experiments as follows: Irradiation of the anomeric proton signals at 2044 Hz, 2008 Hz, 1972 Hz and 1932 Hz resulted in the collapse of the methylene multiplets in the region δ 2.0 - 2.24 and δ 1.65 - 1.80, confirming that these anomeric protons belonged to 2-deoxy sugar units. It was unusual that two of these anomeric protons appeared only as doublets, although their irradiation also led to the collapse of the methylene multiplets in the higher field. The appearance of these two anomeric proton signals of 2-deoxy hexoses as doublets instead of expected doublet of doublets could be explained on the presumption that the molecule assumes such a conformation as to place the axial anomeric protons of the rings S2 and S3 at 90° angles to the equatorial methylene protons, thus giving a value of zero for the coupling constant.

The three anomeric protons of vimose of higher coupling constant (J 8 Hz) were attributable to their axial conformation and related to glycosidic linkages present in the molecule. They, therefore, belonged to the units S2, S3 and S4, suggesting that these L-diginopyranose units were in the $^{1}C_{4}$ conformation[10] and linked through the β-glycosidic linkage. The

small coupling constant of the anomeric proton at δ 5.11 was attributed to its equatorial conformation in the L-diginopyranose unit S1, suggesting that the reducing hexopyranose unit also exists in $^{1}C_{4}$ conformation[10] for it to be α-L-diginopyranose. The above facts are in good agreement with the derivation that vimose (1) is a tetrose of 2,6-dideoxy hexose units.

The MS (CI) of vimose (1) failed to display its molecular ion but contained mass peaks of smaller fragments (Scheme 2) comprising a trisaccharide unit 9 at m/z 450 (3%), a disaccharide unit 12 at m/z 306 (1%), and a monosaccharide unit 10 at m/z 162 (3%) formed by the decomposition pathways in which repeated H-transfers in the oligosaccharide are accompanied by the elimination of the terminal sugars less water.[11] Such a fragmentation gives rise to an ion of the same minimal mass as the molecular ion of the mono-, di- or trisaccharide units. The trisaccharide fragment ion



SCHEME 2

peak (9) at m/z 450 further showed the loss of water, methanol and acetaldehyde common to 3-O-methyl-2,6-dideoxy hexoses,[4] giving fragment ion peaks at m/z 432 (450 - H_2O), 388 (432 - CH_3CHO), 362 (450 - 88), 344 (362 - H_2O) and 330 (362 - CH_3OH). Similarly, the disaccharide unit 12 also showed further losses of these molecules giving fragment ion peaks at m/z 288 (306 - H_2O), 274 (306 - CH_3OH), 262 (306 - CH_3CHO), and 230 (274 - CH_3CHO). The fragmentation of the monosaccharide unit 10 arises from the characteristic fragmentation pattern of the 2,6-dideoxy hexoses reported by Pettit and Brown[11] giving prominent fragment ion peaks at m/z 144 (162 - H_2O), 130 (162 - CH_3OH), 86 9130 - CH_3CHO), 113 (130 - OH) and 95 (113 - H_2O), together with the characteristic ion at m/z 86 (162 - H_2O-CH_3CH=CH-OH).

Another common fragmentation route of oligosaccharides is through a rearrangement involving migration of the methoxyl group to C-1, after the radical-ion fission of the C1 - C2 bond which results in the cleavage of the oligosaccharide.[12] Although the expected fragment ion 11 could not be observed by the above fragmentation, its subsequent fragments by the loss of CH_3CHO gave prominent ion peaks at m/z 290 (334 - CH_3CHO), 246 (290 - CH_3CHO), 317 (334 - OH) and 273 (317 - CH_3CHO). These account for most of the significant peaks in the spectrum that fully support the structure of this tetrasaccharide.

Chemical support for 1's being L-diginotetrose came from its very mild acid hydrolysis[13] at room temperature, which in 3 days exhibited four spots on PC for partially and completely hydrolyzed products. The fastest moving spot had the same mobility as the completely hydrolyzed product, diginose (6), which was taken as reference. The slowest spot (R_{Dig} Ø.47) was identical in mobility to the starting material 1, whereas the third and fourth spots (R_{Dig} Ø.91 and R_{Dig} Ø.6Ø) were presumably for diginobiose (5) and diginotriose (4), respectively, formed by the partial hydrolysis of 1. The reaction was complete in 6 days when the hydrolyzate showed only one spot identical in mobility on PC to 6, thus confirming that vimose (1) is composed of L-diginose units only.

Treatment of 1 with acetic anhydride in pyridine furnished the O-acetyl derivative 2 whose ^1H NMR spectrum (8Ø MHz) was not resolved well enough to give complete structural information. However, it gave prominent signals for four methoxyl groups as two singlets of six protons each at δ 3.45 and δ 3.55, two acetyl groups as two singlets of three protons each at δ 2.Ø5 and δ 2.15, and four secondary methyl groups as two doublets of six protons each (J 6 Hz) at δ 1.2Ø and δ 1.3Ø, respectively. One set of methine proton multiplets of four protons appeared in the region δ 3.1Ø - 3.25. The signals for equatorial and axial methylene protons were obscured by signals of acetyl and secondary methyl groups, respectively.

Further confirmation that 2 is the diacetate of 1 came from very mild alkali hydrolysis (Ø.5% KOH in methanol) of 2 at room temperature. In 3 h the hydrolyzate exhibited three spots on TLC. The fastest spot (R_f Ø.81) showed the same mobility as the starting material 2, while the slowest spot (R_f Ø.16) was identical in mobility with the completely deacetylated product (1), and the third spot of intermediate mobility (R_f Ø.27) is presumed to be the monoacetate 3. In 7 h the hydrolyzate showed only one product which had the same mobility as 1. From the above results it is obvious that in the first stage of hydrolysis the reactive anomeric acetyl group of 2 underwent deacetylation affording the 4-O-acetyl derivative 3, as in the case of orthenthose[4] and ornose.[5]

A negative periodate reaction[14] of 1, which precludes the presence of a vicinal diol grouping in the molecule, and the formation of di-O-acetyl derivative 2 from 1 are in good agreement with the tetrose structure.

In the light of the foregoing evidence, the structure of vimose was established as O-β-L-diginopyranosyl-(1→4)-O-β-L-diginopyranosyl-(1→4)-O-β-L-diginopyranosyl-(1→4)-α-L-diginopyranose (1).

EXPERIMENTAL

General Procedures. Melting points were determined on a Boetius micromelting point apparatus

and are uncorrected. Optical rotations were measured in a 1-dm tube with a JASCO DIP-180 automatic polarimeter. ^{1}H NMR spectra were recorded at 80 MHz with a Varian CFT-20 spectrometer in a proton probe, and the ^{1}H NMR spectrum of vimose was recorded with a Bruker 400-MHz spectrometer. Solutions were ca. 1% in $CDCl_3$ with Me_4Si as the internal standard. The mass spectra (MS) were recorded with a JEOL high-resolution J.M.S.-300 mass spectrometer. The sugars were made visible on TLC with 50% aqueous H_2SO_4. On paper chromatography the sugars were detected with the vanillin - perchloric acid reagent.[15] The lactones were detected on TLC and on PC with NH_2OH - $FeCl_3$ reagent.[16] The adsorbent for TLC was silica gel G (BDH). For column chromatography, silica gel for column (BDH), 60-120 mesh, developed by Ducan's method,[17] was used. Paper chromatography was performed on Whatman No. 1 filter paper using 4:1 toluene - butanol saturated with water as eluant.

Isolation of Vimose (1). - Shade-dried twigs of Orthenthera viminea were extracted by method reported earlier.[18] Mild acid hydrolysis of the isolated glycosides afforded a sugar mixture (2.2 g) which was chromatographed on silica gel (220 g) using 19:1 chloroform - methanol as eluant, collecting 250-mL fractions. Evaporation of fractions 41-48 gave a residue (20 mg). For further purification, the viscous material (20 mg) obtained from a column of

silica gel was rechromatographed on silica gel (20 g). Fractions 12-18, eluted with 19:1 chloroform - methanol (collection of 15-mL fractions), afforded pure amorphous vimose (14 mg), $[\alpha]_D^{25}$ +66.3° (c 0.63, methanol). The product gave a blue coloration (for 2-deoxy sugars) with vanillin - perchloric acid spray,[15] gave positive tests in both xanthydrol[6] and Keller - Killiani[7] reactions, and reduced Fehling's solution. It also gave a negative periodate test.[14] ^{1}H NMR data (400 MHz, $CDCl_3$): δ 5.11 (dd, 1H, J 4 and 1 Hz, H-1 in S1), 5.02 (dd 1H, J 8 and 2 Hz, H-1 in S4), 4.93 (d, 1H, J 8 Hz, H-1), 4.83 (d, 1H, J 8 Hz, H-1), 4.60 - 4.68 (m, 2H, H-5), 4.20 - 4.32 (m, 2H, H-5), 3.80 - 4.15 (m, 4H, H-3), 3.65 (s, 6H, 2 x OCH_3), 3.64 (s, 6H, 2 x OCH_3), 3.20 - 3.56 (m, 4H, H-4), 2.0 - 2.24 (m, 4H, eq. CH_2) 1.65 - 1.80 (m, 4H, ax. CH_2), 1.38 (d, 6H, J 6Hz, 2 x sec. CH_3), 1.33 (d, 3H, J 6Hz, sec. CH_3), and 1.31 (d, 3H, J 6Hz, sec. CH_3); m/z 594 (M^+ not observed), 450 (3%), 432 (5), 388 (5), 362 (1), 344 (1), 330 (3), 317 (1), 306 (1), 290 (100), 288 (1), 274 (2), 273 (10), 262 (2), 246 (3), 230 (1), 204 (30), 178 (6), 162 (3), 156 (15), 148 (14), 144 (4), 133 (6), 130 (3), 113 (11), 103 (21), 97 (2), 95 (3), 86 (7), and 35 (82).

Anal. Calcd for $C_{28}H_{50}O_{13}$: C, 56.56; H, 8.42. Found: C, 56.10; H, 8.83.

Periodate Oxidation of 1. To a solution of 1 (1 mg) in methanol (0.2 mL) was added a solution of

sodium metaperiodate (6 mg) in water (Ø.1 mL), and the mixture was kept for 2 h at room temperature, diluted with water (Ø.4 mL) and evaporated under diminished pressure. The residue was shown to be unreacted 1 by TLC (9:1 chloroform - methanol).

Mild hydrolysis of 1 with acid. To a solution of 1 (6 mg) in 1:1 water - 1,4-dioxane (Ø.5 mL) was added 5 mM H_2SO_4 (Ø.5 mL), and the solution was warmed for 3Ø min at 5Ø °C, cooled and made neutral with freshly prepared barium cabonate. The resulting suspension was filtered, the filtrate was evaporated to dryness under diminished pressure, and the residue was extracted with hot acetone; evaporation of the extract yielded a syrup (6 mg) that exhibited one spot on TLC (92:8 chloroform - methanol) and on PC, having the same mobility on both TLC and on PC as an authentic sample of L-diginose. The product was purified by molecular distillation (high vacuum), yielding a colorless syrup 6 (5 mg), $[\alpha]_D^{25}$ -6Ø.3° (c Ø.72, methanol) that reduced Fehling's solution, gave positive tests for a 2-deoxy sugar in both the xanthydrol[6] and Keller - Kiliani[7] reactions, and did not undergo periodate oxidation.[14] Sugar 6 obtained from the hydrolyzate of 1 was thus identified as L-diginose [6: lit.[9] $[\alpha]_D^{25}$ -65.2° (water)].

Very mild hydrolysis of 1 with acid. To a solution of 1 (2 mg) in methanol (Ø.5 mL) was added Ø.Ø1 M HCl (Ø.5 mL) in 99.5% aqueous methanol, and the

solution was kept at room temperature. After 3 days it showed four spots on PC, two of them showing mobilities identical to 6 (R_{Dig} 1.Ø) and 1 (R_{Dig} Ø.47). The third spot (R_{Dig} Ø.91) and fourth spot (R_{Dig} Ø.6Ø) were presumably the partially hydrolyzed product 5 and 4, respectively. After 6 days the hydrolyzate showed only one spot which had the same mobility as 6. Evaporation of the solution afforded a colorless syrup (2 mg), $[\alpha]_D^{25}$ -62.1° (c Ø.7Ø, methanol) which is comparable with L-diginose [6: lit.[9] $[\alpha]_D^{25}$ -65.2Ø° (water)].

Bromine-water oxidation of 6. A solution of 6 (4 mg) in water (Ø.5 mL) was mixed with bromine (13 µL) and shaken in a stoppered flask in the dark for 24 h at room temperature. The excess of bromine was then removed under diminished pressure, the acidic mixture was neutralized with freshly precipitated silver carbonate to remove Br^- ions, and the suspension was filtered. The filtrate was evaporated to dryness under reduced pressure, yielding a syrupy lactone 7 (3 mg), $[\alpha]_D^{25}$ +27.2° (c Ø.7Ø, methanol, hitherto unreported) showing one spot with NH_2OH - $FeCl_3$ reagent having the same mobility as L-diginono-1,4-lactone on TLC (19:1 chloroform - methanol) and on PC.

Phenylhydrazide 8. A solution of lactone 7 (2 mg) in absolute ethanol (Ø.Ø5 mL) was mixed with freshly distilled phenylhydrazine (Ø.Ø4 mL), and the mixture was heated for 3Ø min at 1ØØ °C. The viscous mass was

cooled and repeatedly triturated with absolute ether (to remove the excess of phenyl hydrazine) yielding a brown powder. The residue (2 mg) crystallized from methanol-ether as colorless needles (1 mg): mp 135 °C; $[\alpha]_D^{25}$ +3.2° (c Ø.54, methanol, hitherto unreported).

Di-O-acetyl vimose 2. A solution of 1 (2 mg) in pyridine (Ø.1 mL) and acetic anhydride (Ø.1 mL) was kept for 48 h at room temperature. The pyridine and the excess of acetic anhydride were then removed under reduced pressure, and the viscous residue was taken up in chloroform and washed in sequence with 2M HCl, 2M Na_2CO_3 solution and water. It was then dried over Na_2SO_4, filtered and evaporated. Di-O-acetyl vimose (2) was obtained as an amorphous residue (2 mg): $[\alpha]_D^{25}$ + 63.3° (c Ø.5Ø, methanol); ^1H NMR data (8Ø MHz): δ5.8Ø (dd, not assigned), 4.75 - 5.Ø (m, 4H, H-5), 3.9Ø - 4.5Ø (m, 4H, H-3), 3.55 (s, 6H, 2 x OCH_3), 3.45 (s, 6H, 2 x OCH_3), 3.1Ø - 3.25 (m, 4H, H-4), 2.15 (s, 3H, OAc), 2.Ø5 (s, 3H, OAc), 1.3Ø (d, 6H, J 6 Hz, 2 x sec. CH_3) and 1.2Ø (d, 6H, J 6 Hz, 2 x sec. CH_3).

Very mild hydrolysis of 2 with alkali. To a solution of 2 (1 mg) in methanol (Ø.2 mL) was added Ø.5% KOH (Ø.2 mL) in 99.5% aqueous methanol, and the solution was kept at room temperature. After 3 h it showed three spots on TLC (99:1 chloroform - methanol). The fastest spot (R_f Ø.81) was identical to 2, and the spots of lower mobilities were identical

to 3 (R_f Ø.27) and 1 (R_f Ø.16). After 7 h the hydrolyzate showed only one spot which had the same mobility as 1.

ACKNOWLEDGMENT

The authors thank Dr. D. K. Kulshreshtha (C.D.R.I., Lucknow) for helpful discussions. One of us (K.N.T.) thanks the C.S.I.R., New Delhi, for the award of a Senior Research Fellowship.

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