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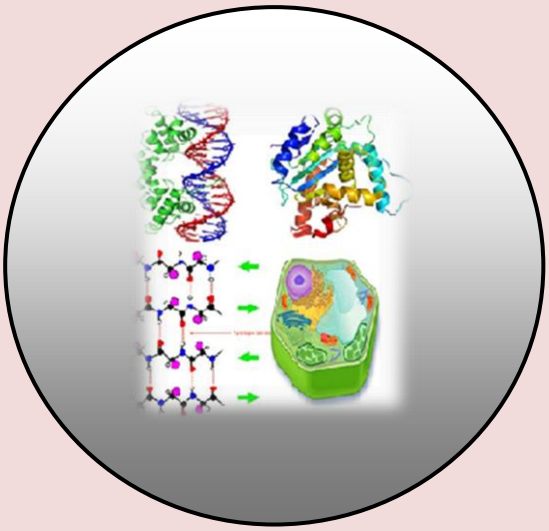

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RESEARCH PAPER

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Isolation and Structure Elucidation of Derivatized Oligosaccharide of 2-deoxy Glucose from *Ichnocarpus frutescens* (Apocyanaceae)

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ABSTRACT

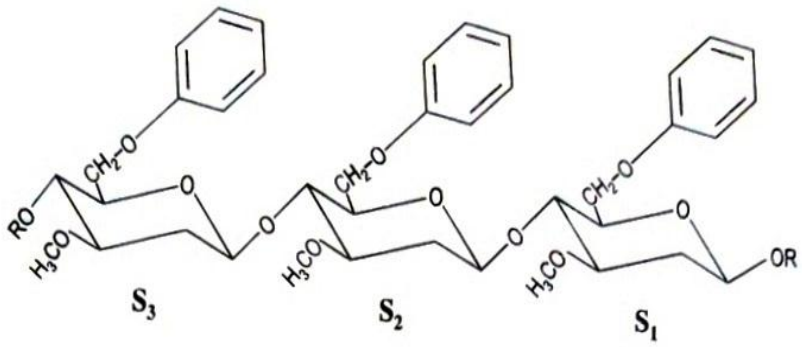
Asclepedaceae and Apocynaceae plant families are a rich source of pregnanes, pregnane glycosides, cardiac glycosides and oligosaccharides of 2-deoxy sugars. In recent times, the importance has been given to sugars of 2-deoxy nature including the oligosaccharides of these sugars. It was proven after the invention of 2-deoxy glucose as an anti-viral agent in COVID-19 times which has come as a medicine for the treatment of this fatal disease and rescue for the society. During the course of investigation, a novel trisaccharide of derivatized 2-deoxy glucose was isolated from the plant Ichnocarpus frutescens and its structure was established by chemical transformation, chemical degradation, 1H, 13C, and COSY NMR and Mass spectrometry as under. 6-phenoxy-3-O-methyl-2-deoxy-β-D-glucopyranosyl(1→4)-6-phenoxy-3-O-methyl-2-deoxy-β-D-glucopyranosyl (1→4)-6-phenoxy-3-O-methy1-2-deoxy-β-D-glucopyranose.
Keywords:* Apocynaceae, Frucose, 2-D Glucose, Covid 19, Oligosaccharide.

INTRODUCTION

Asclepedaceae and Apocynaceae plant families are a rich source of pregnanes (Deepak et al., 1989), pregnane glycosides (Deepak et al., 1997), cardiac glycosides (Deepak et al., 1996) and oligosaccharides of 2-deoxy sugars (Tiwari et al. 1985). These pregnane glycosides were used for tumor and cancer (Yen et al. 2021). They have also showed hypoglycaemic antioxidant and anti-microbial activities (Sayed et al. 2020). Pregnane glycosides isolated from *Mandevilli dardanoi* Lins et al. 2022) showed anti-inflammatory activities. Number of oligosaccharides ornose, orthenthrose and Vimose comprised of 2-deoxy sugars were reported from orthentheraviminiea. In recent researches, the importance of 2-deoxy sugars was investigated by the scientist and 2-deoxy glucose was invented as a medicine for COVID-19 as an anti-viral agent (Kamal et al. 2021). 2-deoxy glucose is essentially a glucose molecule in which the two hydroxyl group has been replaced by the hydrogen. In search for more biologically active oligosaccharides, a derivatized trisaccharide of 2-deoxy glucose was isolated from *Ichnocarpus frutescens*

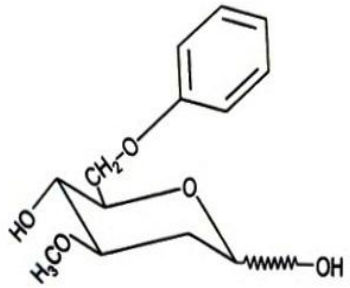
(Apocynaceae) and its structure was established by chemical transformation, chemical degradation, ^{1}H, ^{13}C, and COSY NMR and Mass spectrometry. Substance G, named Frucose (1) $C_{39}H_{50}O_{13}$ (Positive ion FAB-MS: m/z 726[M^{+}] was isolated by repeated column chromatography of chloroform extract of the plant *Ichnocarpus frutescens* (Fam: Apocynaceae) over silica gel and crystallized from. MeOH:CH_2Cl_2 as granular shaped crystals, m.p.168-171°C,$[\alpha]^{D}$+3.570 (c,0.0028, MeOH). Name of the compound Frucose was originated from name of the plant *Ichnocarpus frutescens.* It reduced Fehling solution, and exhibited characteristic positive colour test in Xanthydrol reaction indicating the presence of 2-deoxy sugars in the moiety.

The ^{1}H NMR spectrum of frucose acetate 2 at 300 MHz contained three anomeric proton signals at δ 4.33 (2H, dd, J = 8 and 3Hz) and δ 4.65 (1H, dd. J=8 and3 Hz) as double doublets, along with multiplets for equatorial and axial position of 2-deoxysugar in the region of δ2.27(3H, m, eq.) andδ2.06(3H, m, axial) Thusindicating2 to be the acetate of a trisaccharide composed of three2-deoxy sugarunits. The ^{1}H NMR spectrum of 2 also exhibited singlets of three protons each at δ3.73 (6H) and 3.82 (3H) indicating the presence of three methoxy groups in 2besidsit also showed two three proton singlets at δ2.30 and 2.08 for two acetyl group suggesting it to be a diacetate and leading to the presence of acetyble hydroxyl group in 1. For convenience the three monosaccharide units of 2 were designated as S_1, S_2 and $S_3$from reducing end. The ^{1}H NMR spectrum also contained the signals for H-2. H-3, H-4, H-5 and H-6 at δ 2.06 (3H, m, H-2 ax), δ 2.27 (3H, m, H-2 , eq), δ4.10(3H. m. H-3), δ4.22 (3H, m, H-4), δ4.10 (3H, m, H-5), δ4.02 (6H, dd, J=12 and 3Hz)respectively. The downfield shifting of the protons in the acetate 2 at δ 4.22 from δ 3.83 in S_1 showed the presence of OAc group at H-4 inS_3there by confirming the presence of one free OH group at H-4 of S_3 of the trisaccharide moiety. Similarly the downfield shifting of the anomeric proton of S_1 at δ 4.65 from δ4.42 (1H, dd, J =8 and 3 Hz) in 1 showed the presence of one free hydroxyl group at H-1 of S_1 of the trisaccharide moiety. As the oligosaccharide (1) did not give positive $NaIO_4$oxidation (Sawlewiez et al. 1954), it was proposed that the three methoxy groups may be present at C-3 of each monosaccharide units. The ^{1}H NMR spectrum also contained a multiplet of 15 protons in the aromatic region at δ 6.31 since the compound (1) did not undergo hydrolysis with $NaOCH_3$, (Zemplen et al. 1926)therefore it was assumed that the aromatic protons could be present as three phenyl rings linked to these monosaccharides by ether linkage as phenoxy groups. A close analysis of the 300 MHz ^{1}H NMR spectrum of frucose acetate also helped in ascertaining the configuration of the two ghycosidic linkages. A two proton double doublet at δ 4.33 (2H, dd, J=8 and 3 Hz) could be assigned to two anomeric protons of S_3 and S_2 and the configuration of C-1 in both being identical. Its large coupling constant (dd, J=8 and 3 Hz) was typical of an axial configuration of the anomeric proton of a 2-deoxy hexopyranose in the $^{4}C_1$conforrmation also suggested that units S3 and S2 were linked through a β-D-(1→4) glycosidic linkage (Srivastava et al. 1994). The assignment of these two axial anomeric proton signals is also in agreement with the splitting pattern of their adjacent methylene group with the equatorial protons of the 2-deoxy carbon appearing in the region δ 2.06(2H, mm axial), δ 2.27 (2H, m, eq). A one proton doublet centered at δ 4.65 (1H, dd, J=8 and 2 Hz) was attribuited to the anomeric proton of the sugar S_1 in 2.The larger coupling constant (8 Hz) of this anomeric proton is typical of an axial proton in sugar S_1 in the $^{4}C_1$ (D) conformation.



1 R = H, Frucose

2 R = Ac, Di-O-acetyl frucose



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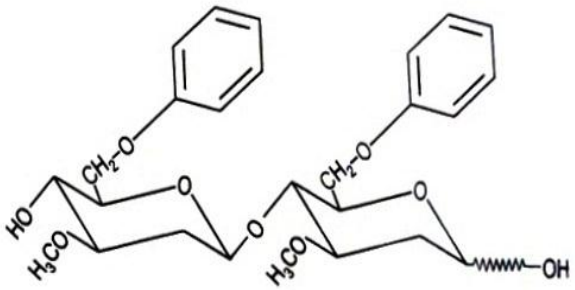

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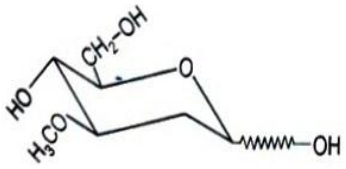

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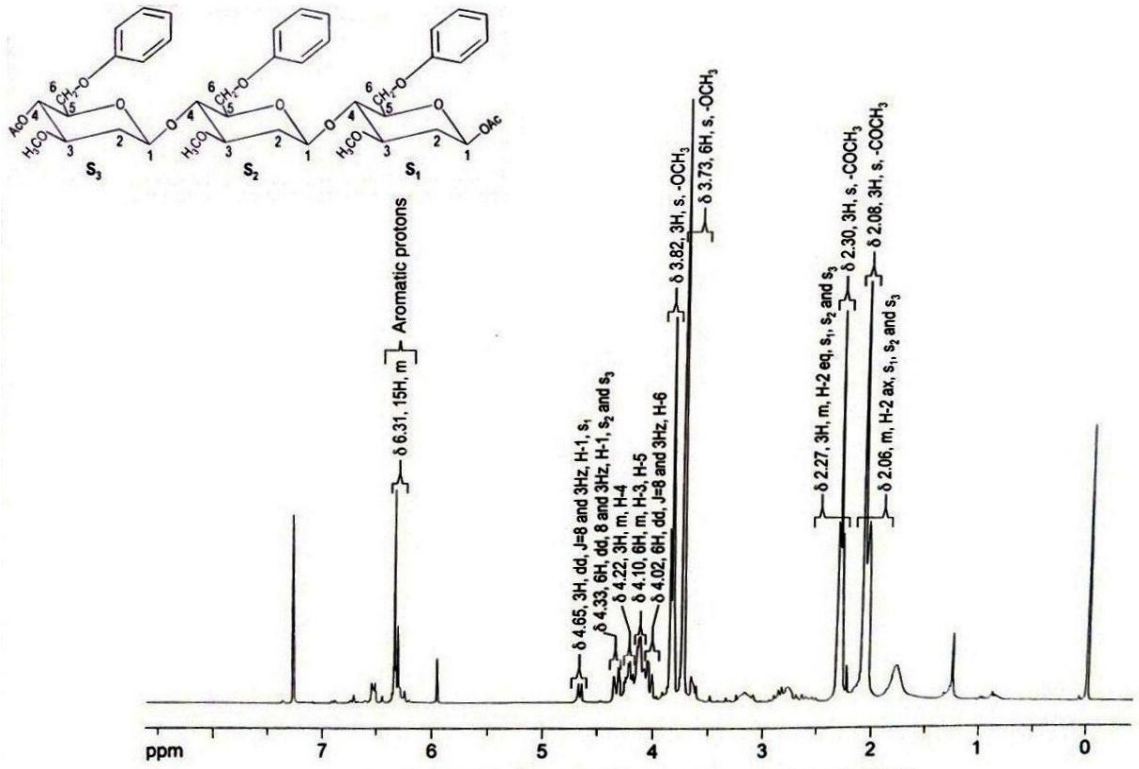
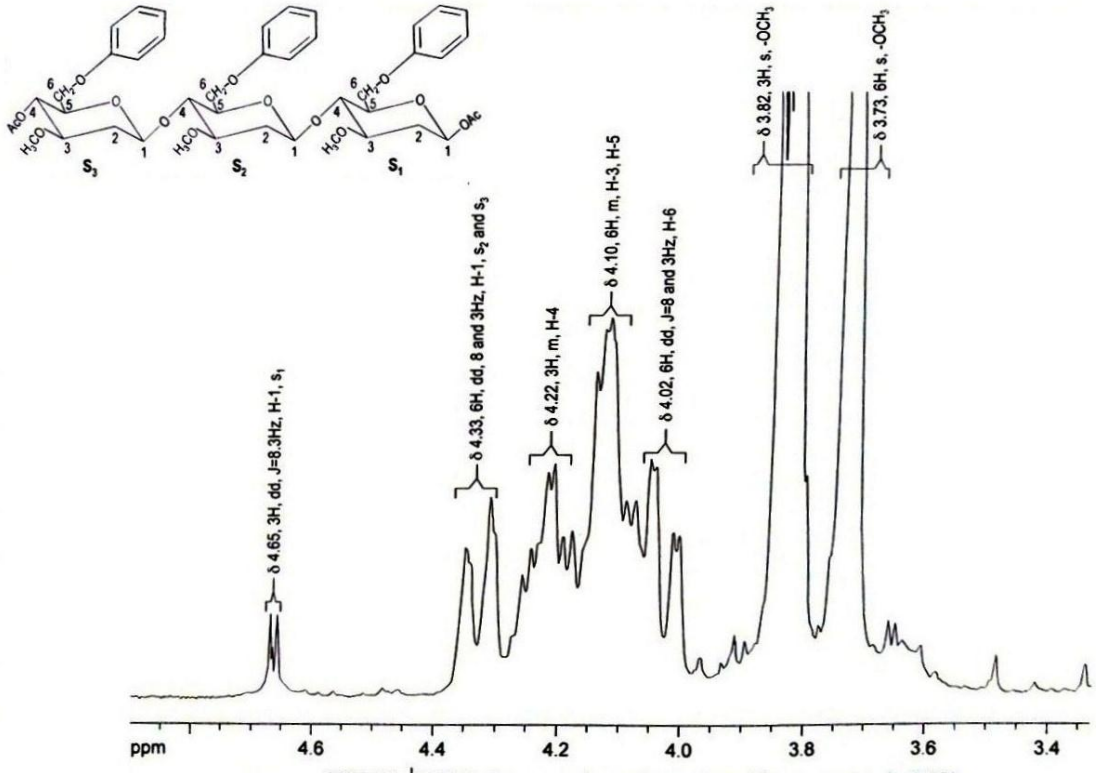
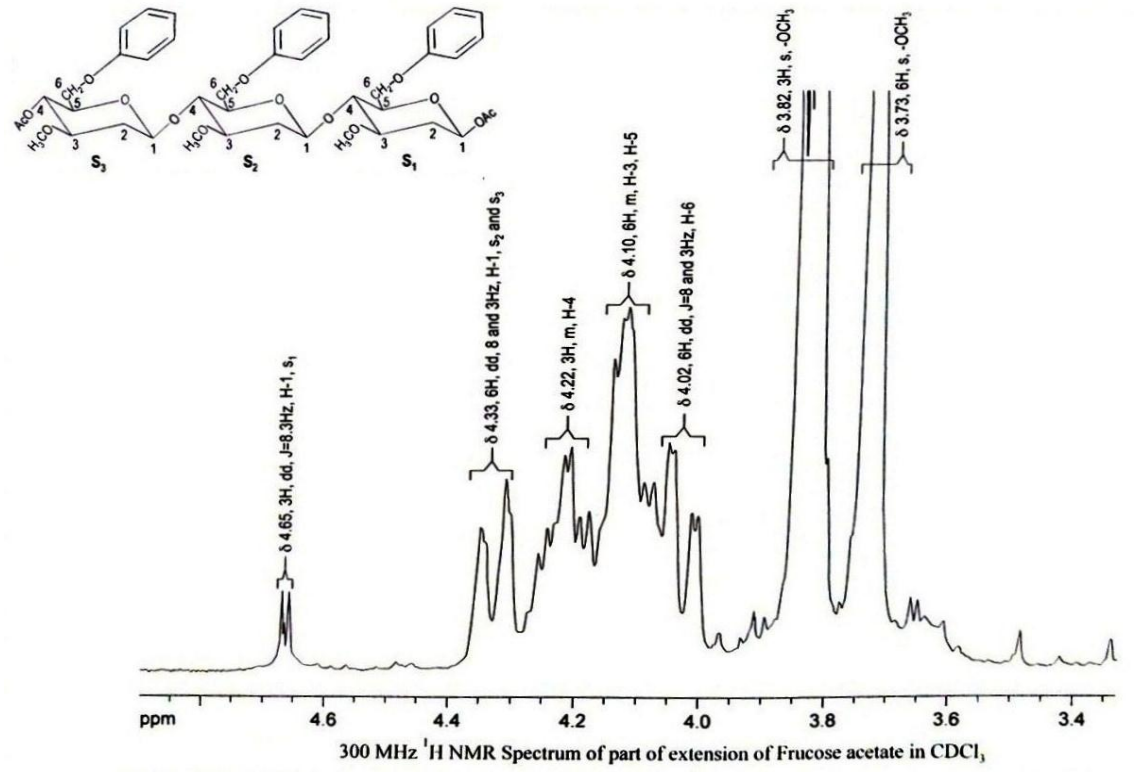
Acid hydrolysis of Frucose



300 MHz ^{1}H NMR Spectrum of Frucose acetate in $CDCl_3$



300 MHz ^{1}H NMR Spectrum of part of extension of Frucose acetate in $CDCl_3$



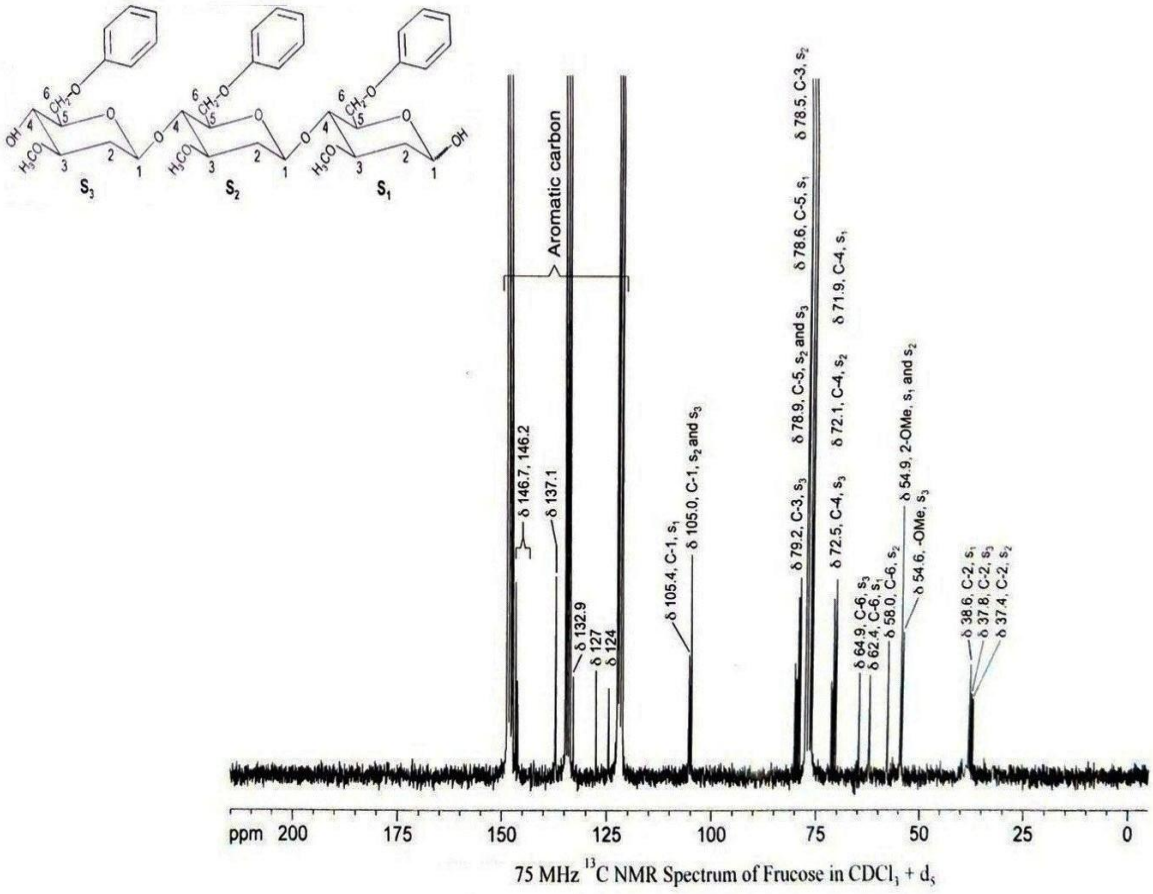
300 MHz ^{1}H NMR Spectrum of part of extension of Frucose acetate in $CDCl_3$

Table 1. 13C NMR shift of Frucose.

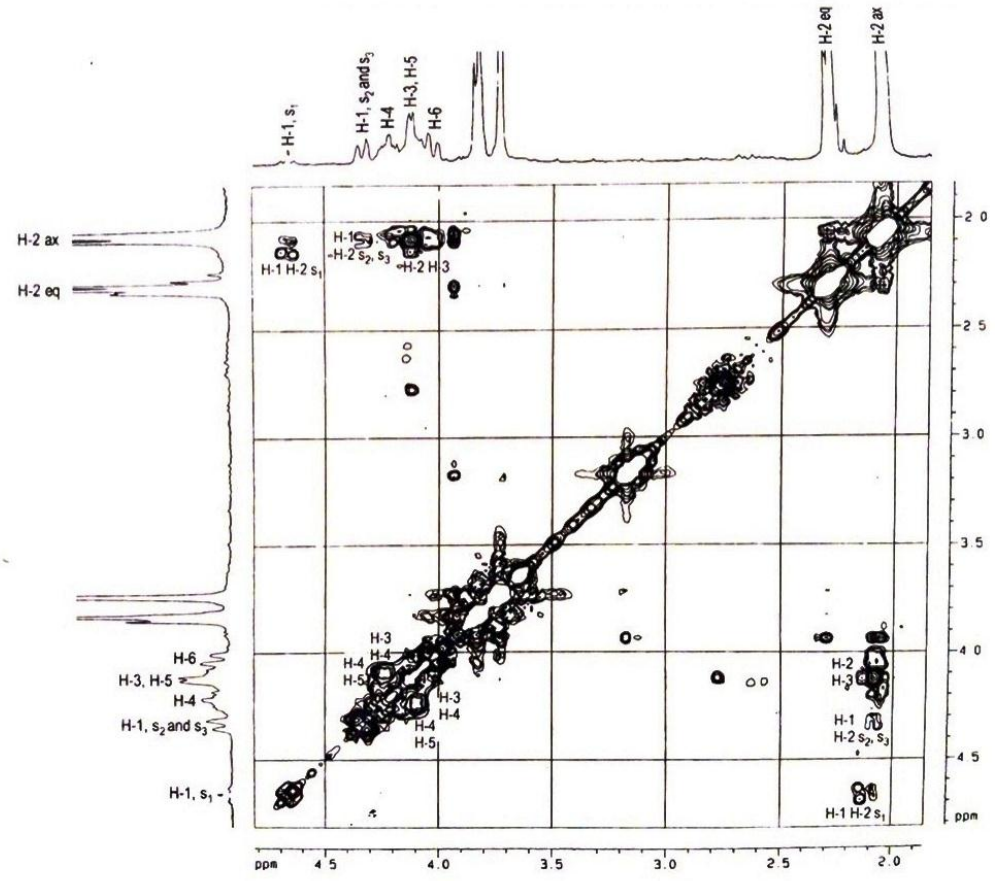
Carbon	Chemical shift of sugar (δ)	Carbon	Chemical shift of sugar and phenyl group (δ)
	Sugar 1		**Sugar 3**
1	105.4	**1**	105.0
2	38.6	**2**	37.8
3	78.3	**3**	79.2
4	71.9	**4**	72.5
5	78.6	**5**	78.9
6	62.4	**6**	64.9
	Sugar 2	**OMe**	**54.9, 54.9, 54.6**
1	105.0		**Phenyl Ring**
2	37.4		147,146, 145.8
3	78.5		137.1, 132.9
4	72.1		127.5, 125
5	78.9		
6	58.0		

A more direct chemical support for the hypothesis that 1 is constituted of three 6-phenoxy-3-O-methyl-2-deoxy pyranoses was provided by the results of its very mild hydrolysis with acid (Rangaswami et al. 1949). Which afforded partially or completely hydrolysed products. Under this condition1 exhibited three spots on PC, TLC within 7 days, the fastest spot had the same mobility as the monosaccharide 6-phenoxy-3-O-methyl-2-deoxy glucopyranose.

The lowest spot was identical mobility as the starting material 1whereas third spot was presumable disaccharide unit formed by the partial hydrolysis of 1. This hydrolysis was complete in 15 days when the hydrosylate contained only one sugar $[\alpha]_D$-24.8°. It gave positive Xanthdrol test and showed negative $NaIO_4$ oxidation, and did not undergo hydrolysis with sodium methoxide. When it was treated with H_2/Pd, it gave compound (5) (Ferrer et al. 1965) which was identical mobility with 3-O-methyl-2-deoxy-β-D-glycopyranose. The structure of Frucose (1) was further supported by data of its ^{13}C NMR which was in close conformity with the ^{1}H NMR spectral data of 1. Besides the presence of three anomeric carbon signals at δ 105 (2C) and 105.4 (1C), it contains signal for three methoxy carbon signals which appeared at δ 54.9(2C) and δ 54.6(1C). It also contained the signal for ring carbons along with aromatic carbon (table 1).

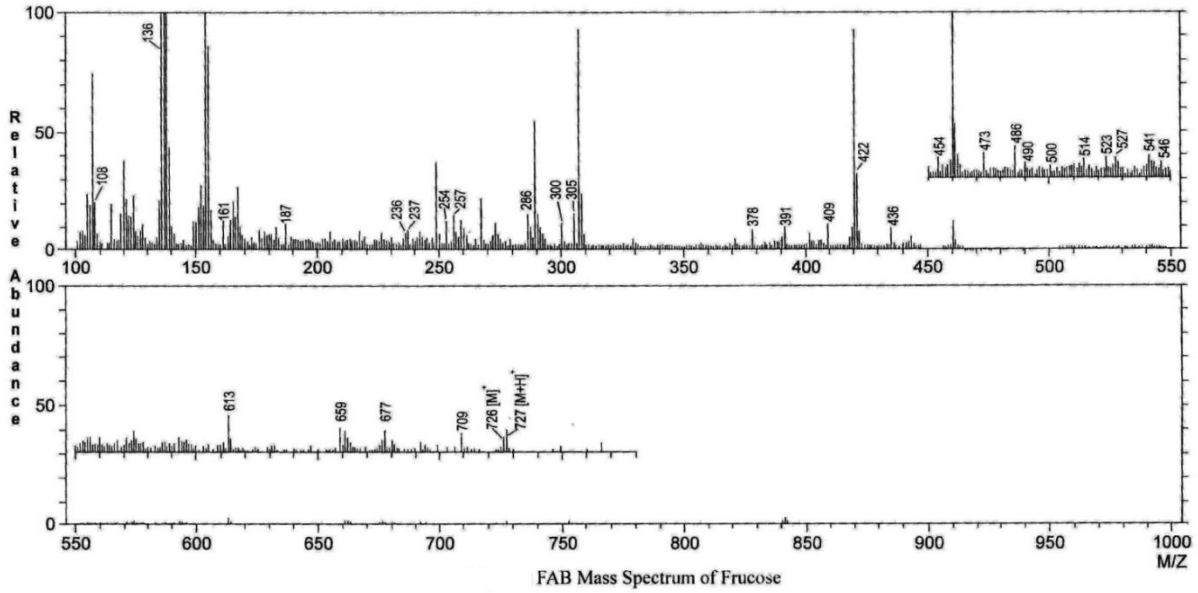


All the ^{1}H NMR assignments for the ring protons of monosaccharide unit moiety of Frucose (1) were confirmed by 2D HOMOCOSY experiment. The 2D HOMOCOSY spectrum of 2 showed the connectivity of anomeric proton at δ 4.33 and 4.65 with H-2/ equatorial and axial protons at δ 2.27 and 2.06 respectively.



300 MHz 2D ^{1}H-^{1}H COSY Spectrum of Frucose acetate in $CDCl_3$

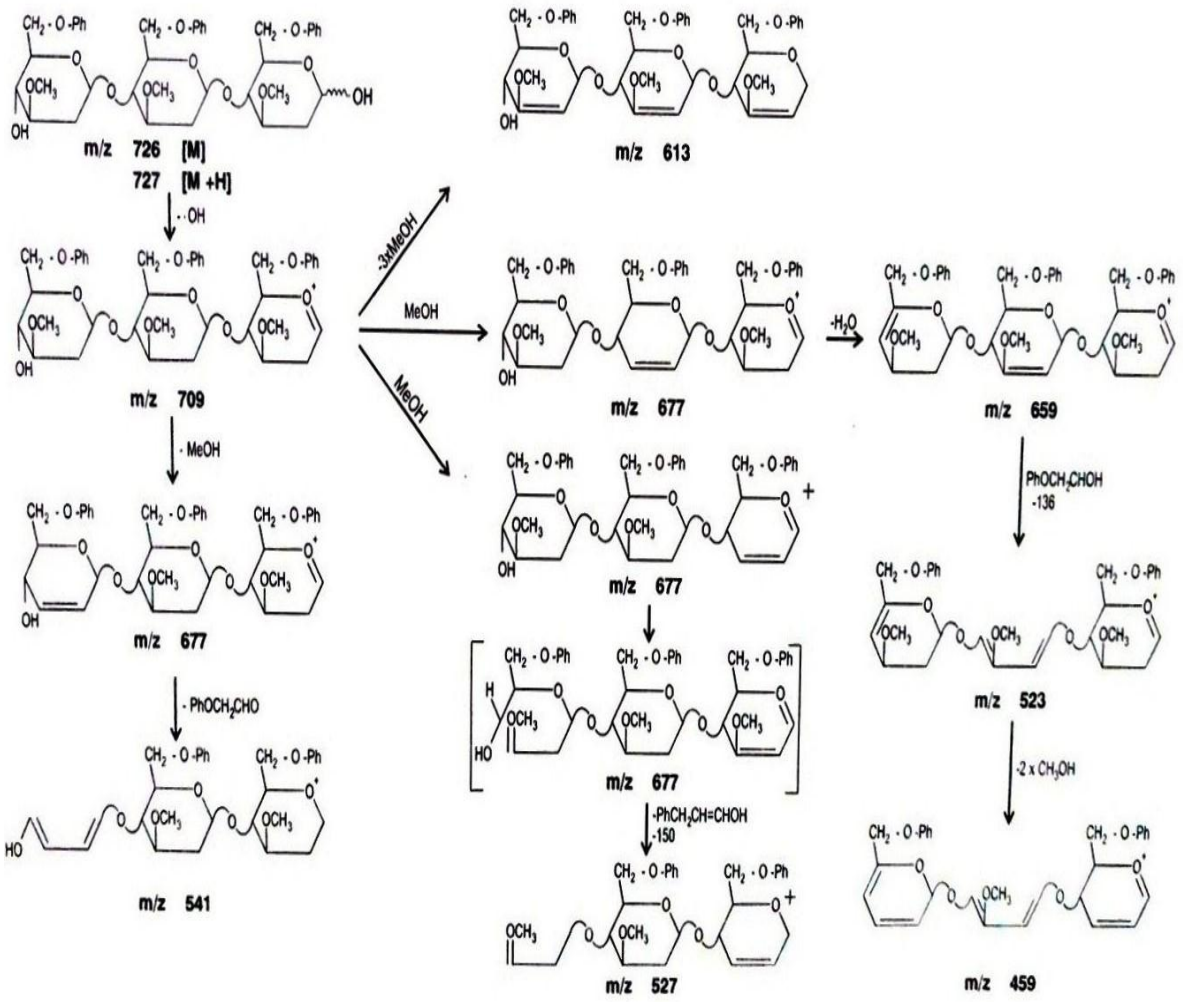
The FABMS of 1showed the highest mass ion peak at m/z 727 and 726 which could assigned for M+1 and M^{+} respectively. Other important information obtained from the FABMS of 1 was through the ion peak at m/z 709 which corresponded to (trisaccharide-OH).



FAB Mass Spectrum of Frucose

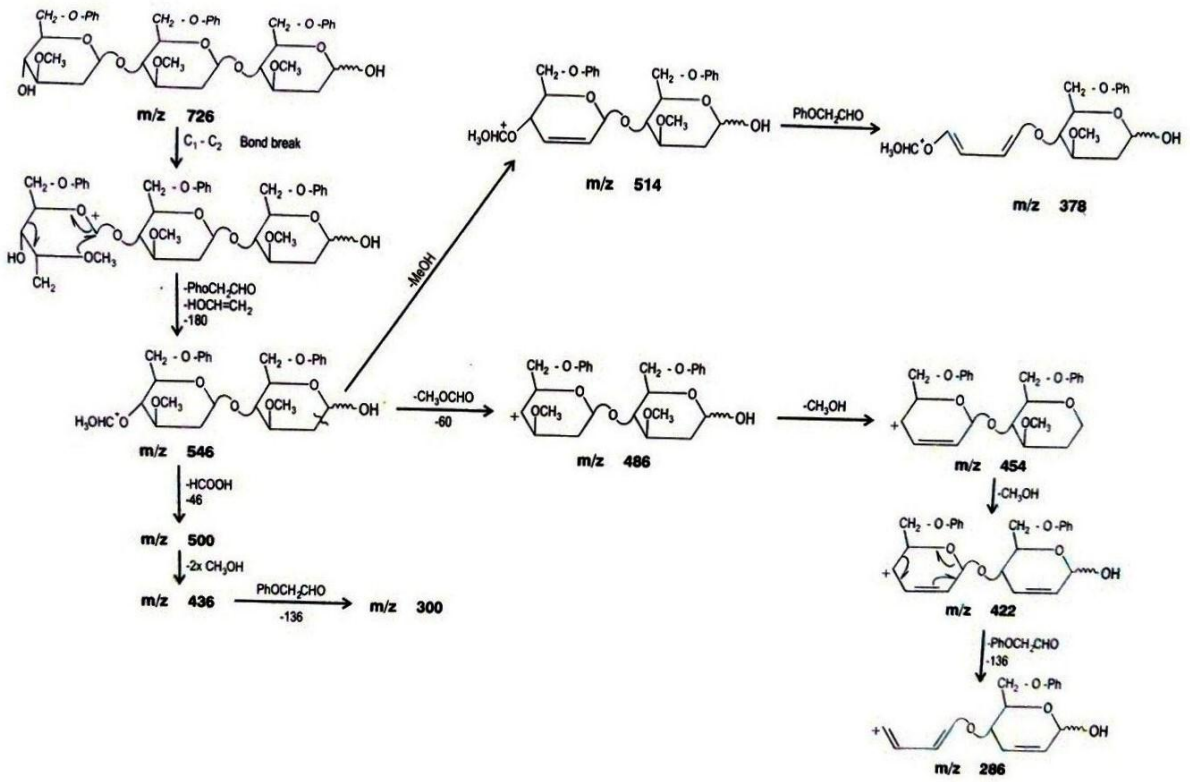
Another important fragments of trisaccharide was originated from fragment ion at m/z 709 by consequent loss of methanol and water molecules to give the mass ion peak at m/z 677 [709-CH_3OH], 659 [677-H_2O] respectively.

The fragmented ion peak at m/z 523 was attributed to [659-$PhOCH_2CHO$]. Which was followed by further loss of two methanol molecules to yield fragment ion peak at m/z 459 (Scheme-1).



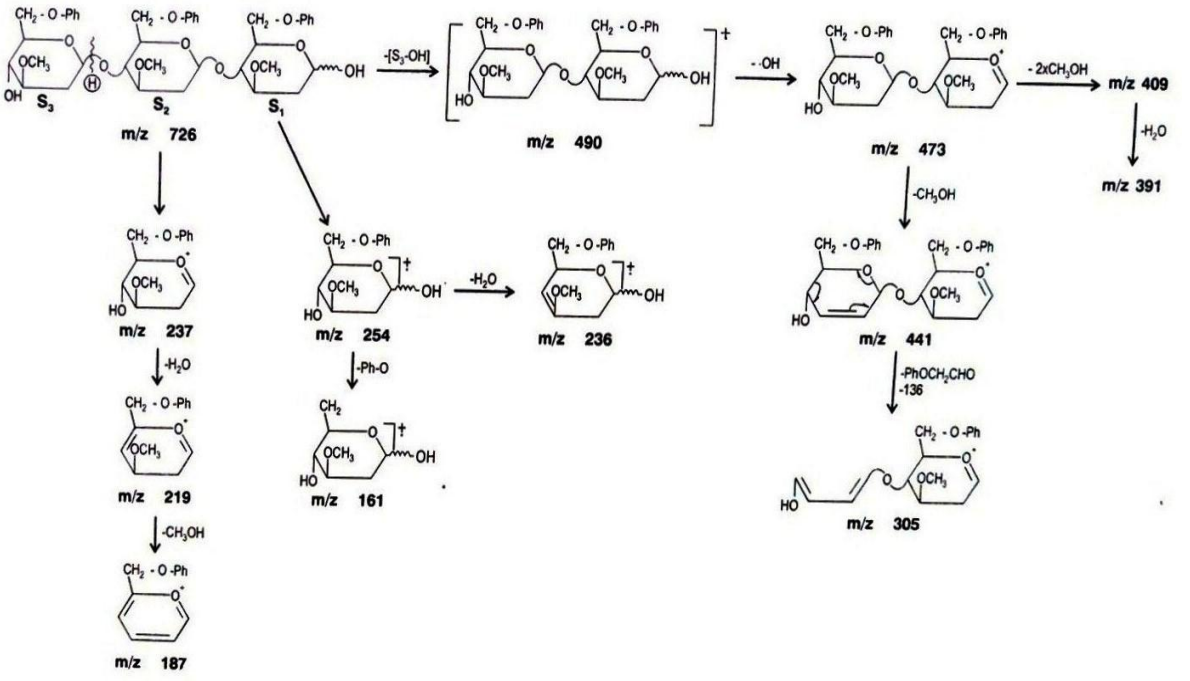
Scheme - 1: FAB Mass Fragments of Frucose

The mass ion fragment at m/z 546 [trisaccharide-C1oH1203] originated by the redical ion fission of C-1—C-2 bond of S3 and subsequent migration (Brownet al. 1971, Oliveret al. 1988) of methoxy group attached to C-3 of S3 to C-1 of the same unit. It subsequently fragments to give ion peaks of m/z 486 [546- CH_3OCHO], 454[486-CH30H], 422 [454-CH3011], 286 [422-$PhOCH_2CHO$] (Scheme- 2).



Scheme - 2: FAB Mass Fragments of Frucose

The FABMS also contains the mass fragments for disaccharides unit at m/z 490 (Scheme- 3).



Scheme - 3: FAB Mass Fragments of Frucose

The important mass ion fragments obtained by fragmentation of these disaccharide were at m/z 473[490-OH], 441[490-CH3OH], 305 [441-Ph-O-CH2CH0], 409[473-2xMeOH], 391[409-H_2O]. The FABMS also contains the monosaccharide mass fragments at m/z 254 which further fragmented to give the characteristic mass fragments at m/z 236 and m/z 161 which were due to loss of H_2O and a phenoxy group respectively. Thus the structure of monosaccharide was established as 6-phenoxy-3-O-methyl-2-deoxy glucose. The only available position for interglycosidic linkage was at C4 thus making a 1→4 glycosidic linkage which was confirmed by the ^{1}H NMR of Frucose acetate. In light of the forgoing evidences the structure of 1 was established as 6-phenoxy-3-Omethyl-2-deoxy-β-D-glucopyranosyl(1→4)-6-phenoxy-3-O-methyl-2-deoxy-β-Dglucopyranosyl(1→4)-6-phenoxy-3-O-methy1-2-deoxy-β-D-glucopyranose.

EXPERIMENTAL

General Procedures

All melting points were recorded on Boetius, micro melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 digital polarimeter in 1 cm tube. ^{1}H NMR, 13C NMR and 2D spectra were recorded with AVANCE DRX 300 MHz, 200 MHz Bruker spectrometers in $CDC1_3$, d; and d6 using TMS as internal standard. FAB mass spectra were recorded with JEOL mass spectrometer model JMS-SX-102 with DA-6000 Data system and JEOL Mass spectrometer D-300 with IMA-2000 Data system respectively. Pure compounds were visualized on TLC with 50% aq H_2SO_4 reagent and on PC with vanillin-perchloric acid Partridge reagents. The absorbent for TLC was silica gel G (SRL) and for CC, silica gel for column (SRL 60-120 and 120-200 mesh) developed by Duncan's method, PC was performed on whatman No.-1 filter paper using solvent system C6115-CH3 : BuOH (4:1) saturated with water.

Experimental

Substance G

Substance G was isolated from fraction 59-90 of CC-7 and fraction 17-32 of CC-8 (103.7 mg). Crystallized from CH_2Cl_2: Me0H, (95:5), mp. 168-171°C $[\alpha]_D$ -3.5° (c, 0.0028. MeOH).

It responded positively to Xanthydrol and Keller-Killiani reactions, gave blue colouration in Vanillin-perchloric acid spray reagent. It reduced the Fehling solution. For elemental analysis substance I was dried over Pal-1m at 100°C and 0.1 mm pressure.

		%C	%H
$C_{39}H_{50}O_{13}$	**Calcd**	64.46	6.88
	Found	64.45	6.88

^{1}H NMR: ^{1}H (δ ppm from TMS in $CDC1_3$+C_5D_5N (d5).

1.88 (3H, m), 2.18 (3H, m), 3.45 (6H, s), 3.65 (3H, s), 3.67 (3H, m), 3.74 (3H, m): 3.83 (3H, m), 3.90 (6H, dd, J = 12 and 3Hz), δ 4.28 (2H, dd, J = 8 and 3Hz) δ 4.42 (1H, dd, J = 8 and 3Hz), 6.54 (15H, s).

^{1}H NMR: ^{1}H (δ ppm from TMS in $CDC1_3$ + DMSO (d6)

1.64 (3H, m), 1.84 (3H, m), 2.60 (6H, s), 3.31 (3H, s), 3.48 (3H, m), 3.59 (3H, m), 3.66 (3H,m), 3.76 (3H, s), 3.81 (3H, m), 3.85 (3H, s), 4.07 (3H, d, J = 7.4Hz), 6.35 (9H,$), 6.46 (6H, s).

^{13}C NMR: ^{13}C (δ ppm from TMS in $CDCI_3$ + C_5D_5N (d5)

146.7, 146.2, 137.1, 132.9, 127.5, 124.5, 105.4, 105.0, 79.2, 78.9, 78.6, 78.5, 78.3, 72.5, 72.1, 71.9, 64.9, 62.4, 58.0, 54.9, 54.6, 38.6, 37.8 and 37.4.

FABMS m/z : 727, 726, 709, 677, 659, 613, 546, 541, 527, 523, 514, 500, 490, 486, 473, 554, 441, 436, 422, 409, 391, 378, 305, 300, 286, 257, 254, 236, 237, 219, 290, 237, 187, 136, 108 and 107.

The above spectral data along with the diagnostic colour reactions are consistent with its identification of novel oilgasaccharide containing three 2-deoxy sugars unit phenoxy ring is connected at 6 positions in 2-deoxy sugar as ether linkage.

Mild Hydrolysis of 1 with Acid

To a solution of crystalline 1 (15 mg) in 80% aq. 1, 4-dioxane (2.5 ml) 0.1 N H_2SO_4 (2.5 ml) was added and the solution was kept at the room temperature. After 7 days, the reaction mixture exhibited three spots on PC TLC. The fastest spot had the same mobility as the monosaccharide 6-phenoxy-3-0-methyl-2-deoxy glucopyranose (3): (TLC). The lowest spot was identical mobility as the starting material 1. Whereas third spot was presumable disaccharide unit formed by the partial hydrolysis of 1. The hydrolysis was complete in 15 days when the hydrolysate contained only one sugar. Dioxane was then removed under reduced pressure. The aq. hvdrolysate was neutralized with freshly prepared $BaCO_3$, filtered and concentrated under reduced pressure to afford chromatographically pure derivatized reducing sugar as syrup identified as derivatized sugar 6-phenoxy-3-O-methyl-2-deoxy glucopyranose (3) and (8.5mg) $[\alpha]_D$ -24.8°. It gave positive Xanthydrol test and did not undergo hydrolysis with sodium methoxide. It was treated with H_2/Pd, it gave compound (5) (2.5 mg), which was identical movability with 3-O-methyl-2-deoxy-β-D-glucopyranose by comparison with polarity of starting material compound (3) by PC and TLC.

Acetylation of 1

Crystalline 1 (22.5 mg) was acetylated with Ac_2O (2 ml) in pyridine (2 ml) 100°C for 1 hour. Pyridine and excess of Ac_2O was then removed under reduced pressure. In viscous residue taken in $CHC1_3$ (5 ml) was washed in sequence with 2N HC1 (1x5 ml), Ice-cold 2N $NaHCO_3$ (2x5 ml) and finally with H_2O (2x5 ml). $CHCl_3$ layer was dried over anhyd. Na_2SO_4 filtered and evaporated to dryness yielding amorphous di-O-acetyl frucose2 (23mg).

^{1}H NMR: ^{1}H (δ ppm from TMS in $CDC1_3$)

2.06 (3H, m, H-2 ax S_1, S_2, S_3), 2.08 (3H, S, $OCOCH_3$), 2.30 (3H, S, $OCOCH_3$), 2.27 (3H, m, H-2, eq S_1, S_2, S_3), δ 3.73 (6H, s, OCH_3), δ 3.82 (3H, s, OCH_3) 4.02 (6H, dd, J = 12, 3Hz, H-5), 4.10 (6H, m, H-3, and H-5), 4.22 (3H, m, H-4), 4.33 (6H, dd, J = 8 and 3Hz, H-1, S2 and S3), 4.65 (3H, dd, J = 8 and 3 Hz, H-1, S_1), 6.31, 15H, m, Aromatic protons.

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