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SULFATED GLYCOSIDES. STRUCTURAL PROOF FOR SALVADOSIDE THROUGH COMPARISON WITH THAT REGIOSELECTIVELY SYNTHESIZED FROM D-GLUCOSE

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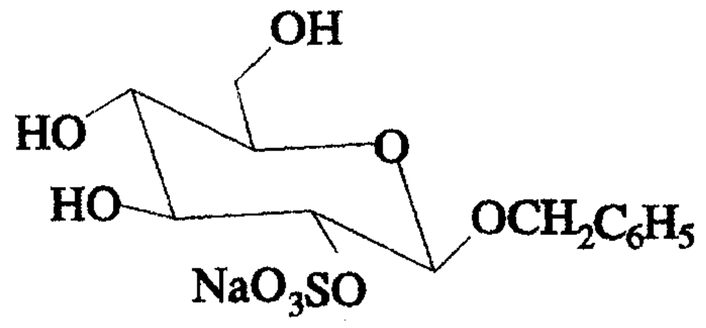
مركب السلفادوزيد صوديوم (١-أ-بنزيل-بيتا-جلوكوبيرانوزيد-٢-كبريتات) الذى تم فصله مؤخرا من نبات السواك والمعروف فى الطب الشعبى باستخدامه فى علاج التهابات المعدة ، أمكن تحضيره معمليا فى أربعة خطوات من الجلوكوز وذلك بالإضافة إلى بعض النظائر الأخرى.
وقد أثبتت مقارنة المركب الطبيعى بالمركب الذى تم تخليقه من حيث الصفات الكروماتوجرافية بجانب التحاليل الطيفية ومطياف الكتلة أنهما متطابقان تماما مما يعد تأكيدا للتركيب البنائى للمركب الطبيعى.

Salvadoside; sodium (1-O-benzyl-ß-D-glucopyranoside-2-sulfate) [1], a recently isolated sulfated glycoside from the Egyptian plant Salvadora persica L. used in the treatment of gastritis in folk medicine, has been synthesized in four steps from glucose. The route adopted also allows regioselective synthesis of structural isomers of the target compound

INTRODUCTION

Sulfated glycosides are abundant in natural biopolymers, especially in sulfated glycosaminoglycans such as heparan sulfate, chondroitin sulfate, keratan sulfate, and dermatan sulfate. Together with sulfated glycosphingolipids these play a major role in cell adhesion, signal transduction and inflammation[1]. Simple sulfated carbohydrates, on the other hand, have so far largely evaded closer examination. A major issue in the chemical synthesis of complex sulfated carbohydrate derivatives is the achievement of regioselective sulfation. In this paper we report our findings on such an event in the case of glucose sulfation.

Salvadoside; [sodium (1-*O*-benzyl-ß-D-glucopyranoside-2-sulfate)], **1** is a recently isolated sulfated glycoside from the Egyptian plant *Salvadora persica* L.[2] that is used in the treatment of gastritis in folk medicine[3]. Its structure was elucidated on the basis of spectroscopic data. The successful synthesis of this compound as a free acid, together with its structural isomers constitute a proof of the original structural assignment.



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EXPERIMENTAL

General procedures

Acetone was dried by distillation from potassium carbonate. Pyridine was dried with solid KOH. Benzyl alcohol was distilled prior to use. All other reagents were used as obtained from commercial suppliers. Reactions were monitored by thin-layer chromatography on pre-coated aluminum-backed silica gel 60 F_{254} plates from Merck. NMR spectra were recorded on a

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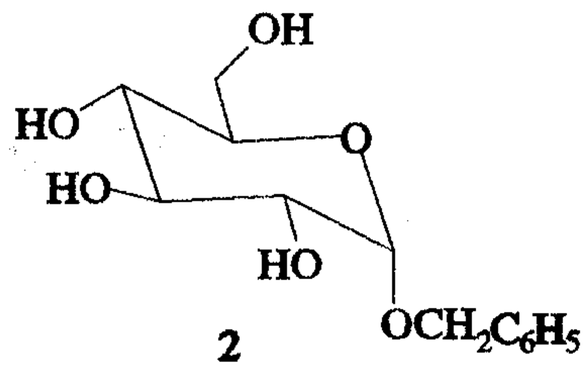
Bruker AM-200 or a Jeol GSX 400 instrument. Optical rotations were determined on a Perkin-Elmer 241B digital polarimeter in a 1 dm/1 mL cell.

Benzyl α- and ß-D-glucopyranosides 2,3[4]

D-Glucose (8.14 g, 45.2 mmol) was suspended in 70 mL benzyl alcohol. The mixture was warmed to 95°C, then a saturated solution of anhydrous HCl in benzyl alcohol (5 mL) was added. The suspended material dissolved, and the resulting solution turned dark yellow after heating for 6 h. The solution was filtered through glass wool into 400 mL of vigorously stirred dimethyl ether. The precipitated mixture of glycosides **2** and **3** (8.0 g, 66%) was filtered, washed thoroughly with ether and air-dried. ^{13}C-NMR (DMSO) δ 138.2 (Ar-Cl), 128.2 (Ar-C2,6), 127.6 (Ar-C3,5), 127.4 (Ar-C4), 102.1 (C-1 ß-Glc), 97.9 (C-1, α-Glc), 77.0 (C-3, ß-Glc), 76.8 (C-5, ß-Glc), 73.6 (C-2, ß-Glc), 73.4 (C-3 α-Glc), 73.1 (C-2 α-Glc), 72.0 (C-5 α-Glc), 70.4 (C-4 ß-Glc), 70.2 (C-4 α-Glc), 69.5 (Ar-CH_2-O ß-isomer), 67.7 (Ar-CH_2-O α-isomer), 61.2 (C-6 ß-Glc), 61.0 (C-6 α-Glc).



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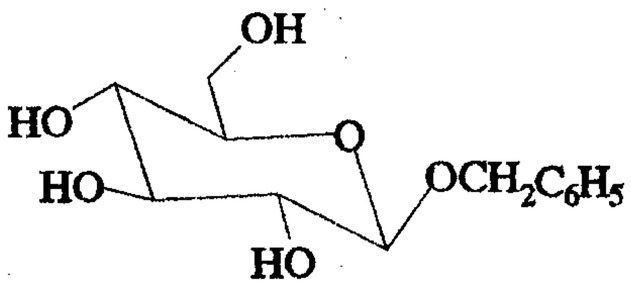

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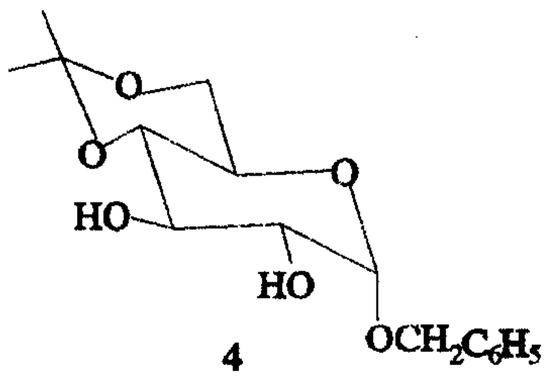
Benzyl 4,6-O-isopropylidene-α-D-glucopyranoside 4 and benzyl 4,6-*O*-isopropylidene-ß-D-glucopyranside 5

The crude glycosides **2,3** (8.0 g, 30 mmol) was suspended in a mixture of acetone (200 mL) and 2,2-dimethoxypropane (80 mL). *p*-Toluenesulfonic acid monohydrate (700 mg, 3.7 mmol) was added and the mixture was stirred for 3 h at rt. during which time a clear yellow solution was formed. The solvent was evaporated under reduced pressure at room temperature. The residue was dissolved in dichloromethane, washed with dilute sodium bicarbonate, water and brine, dried over anhydrous sodium sulfate and filtered. Rotary evaporation of the solvent under reduced pressure gave a light brown oil which was purified by column chromatography on silica gel using 2.5% methanol/chloroform as the eluant to afford a mixture of the two anomers **4** and **5** (7.0 g, 76%). A portion of the mixture (1.2 g) was subjected to MPLC on silica gel using hexane-ethyl acetate (1:1) as the eluant. The two anomers could be isolated: **4** (800 mg, 67%) and **5** (250 mg, 21%). Data for **4**: yellow oil, $[\alpha]_D$= +80.3° (*c*= 0.78, MeOH). R_f 0.16 (*n*-hexane-EtOAc), ^{1}H-NMR (CD_3OD) δ 1.36 (3H, s), 1.48 (3H, s), 3.4-3.8 (4H, m), 4.56 and 4.69 (2H, 2d, J= 11.7 Hz), 4.89 (1H, d, J= 4.0 Hz), 7.2-7.5 (5H, m). ^{13}C-NMR (CD_3OD) δ 20.1, 30.3, 64.1, 65.7, 71.5, 73.0, 74.9, 76.1, 100.9, 101.5, 129.6, 130.0 (2C), 130.1 (2C), 139.6. FAB MS m/z 333 (M+Na). HRMS 333.1319, calculated for $C_{16}H_{22}O_6Na$ 333.1314. Data for **5**: Yellow oil, $[\alpha]_D$= -75.4° (*c*= 0.21, MeOH). R_f 0.24 (*n*-hexane-EtOAc, 1:1). ^{1}H-NMR (CD_3OD) δ 1.38 (3H, s), 1.49 (3H, s), 3.2-3.9 (4H, m), 4.43 (1H, d, J= 7.7 Hz). 4.62 and 4.85 (2H, 2d, J= 11.7 Hz), 7.2-7.5 (m, 5H). ^{13}C-NMR (CD_3OD) δ 20.1, 30.2, 63.9, 69.2, 72.9, 75.5, 75.7, 76.7, 101.5, 104.9. 129.5, 129.8 (2C), 130.1 (2C), 139.6. FABMS m/z 333 (M+Na). HRMS 333.1347, calculated for $C_{16}H_{22}O_6Na$ 333.1314.

Benzyl-ß-D-glucopyranosyl-2-sulfate 1 and Benzyl-ß-D-glucopyranosyl-3-sulfate 7

Compound **5** (48 mg, 0.155 mmol) was



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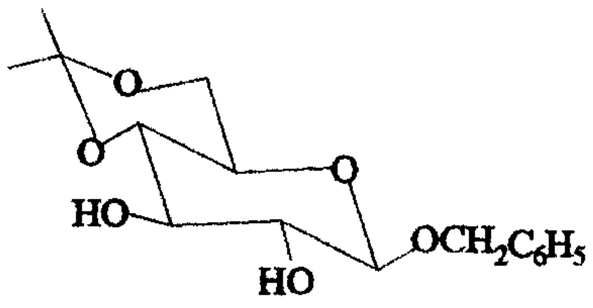

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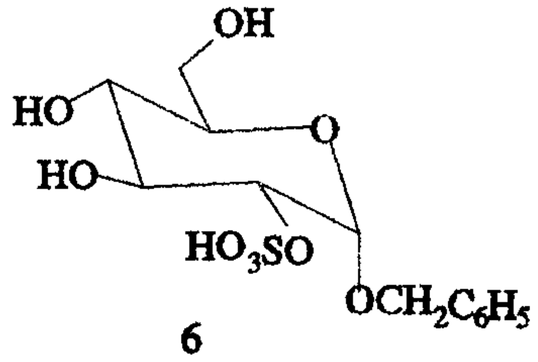

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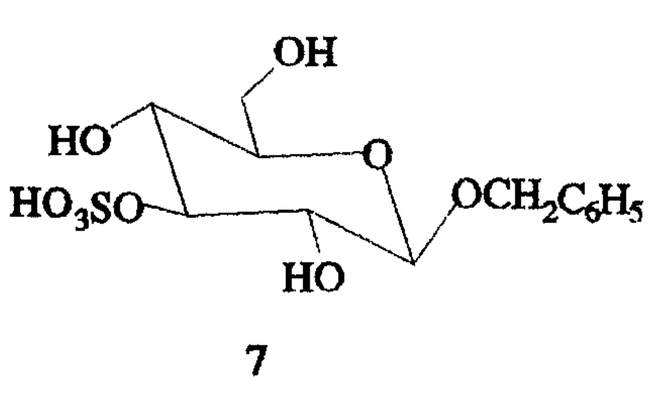

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dissolved in 2 mL pyridine and stirred with sulfur trioxide-pyridine complex (24 mg, 0.151 mmol) for 3 h at rt. The reaction mixture was then transferred to a column packed with silica gel and eluted successively with 5% and 15% methanol in chloroform. The obtained mixture was dissolved in methanol and left for three days at room temperature. A mixture of two more polar compounds was produced and subjected to preparative TLC using 25% methanol/ chloroform as the eluting system to produce the two desired sulfated benzyl glucosides **1** and **7** in equal amounts (22 mg, 41% each). Data for the free acid of **1**: Yellow oil, $[\alpha]_D$= -13.2° (*c*= 0.17, MeOH), R_f 0.11 ($CHCl_3$-MeOH, 75:25). ^{1}H-NMR (CD_3OD) δ 3.2-4.0 (m, 5H), 4.14 (1H, dd, J= 7.7, 8.8 Hz), 4.51 (1H, d, J= 7.7 Hz), 4.71 and 4.93 (2H, 2d, J= 12.1 Hz), 7.1-7.5 (5H, m). ^{13}C-NMR (CD_3OD) δ 63.6, 72.4, 72.5, 78.4, 78.5, 82.3, 102.0, 129.2, 129.7 (2C), 129.9 (2C), 139.9. FAB MS m/z 395 (M+2Na). HRMS 395.0379, calcd for $C_{13}H_{17}O_9SNa_2$ 395.0389. Data for **7**. Colorless oil. $[\alpha]_D$= -15.6° (*c*= 0.17, MeOH). R_f 0.19 ($CHCl_3$-MeOH, 75:25). ^{1}H-NMR (CD_3OD) 3.3-4.0 (5H, m), 4.22 (1H, t, J= 8.8 Hz), 4.45 (1H, d, J= 9.0 Hz), 4.67 and 4.92 (2H, 2d, J= 11.2 Hz), 7.2-7.5 (5H, m). ^{13}C-NMR 63.3, 71.2, 72.6, 74.5, 78.3, 86.4, 103.7, 129.5, 130.0 (2C), 130.0 (2C), 139.7 FAB MS m/z 395 (M+2Na). HRMS 395.0420, calcd. for $C_{13}H_{17}O_9SNa_2$ 395.0389.

Benzyl-α-D-glucopyranosyl-2-sulfate 6

Compound **4** (44 mg, 0.142 mmol) was dissolved in 2 mL pyridine and stirred with sulfur trioxide-pyridine complex (22 mg, 0.138 mmol) for 24 h at rt. The reaction mixture was transferred to a column of silica gel and eluted first with 5% methanol/chloroform, then with 15% methanol/chloroform to afford compound **6** (33 mg, 67%). Dark yellow oil. $[\alpha]_D$= +22.8° (*c*= 0.28, MeOH). R_f 0.06 ($CHCl_3$-MeOH, 75:25). ^{1}H-NMR (CD_3OD) δ 3.3-4.0 (5H, m), 4.17 (1H, dd, J= 3.6, 9.9 Hz), 4.55 and 4.76 (2H, 2d, J= 11.7 Hz), 5.29 (1H, d, J= 3.6 Hz), 7.2-7.5 (5H, m). ^{13}C-NMR 63.2, 71.7, 72.7, 73.8, 74.4, 79.7, 98.8, 129.4, 129.8 (2C), 130.0 (2C), 139.9 FAB MS 395 (M+2Na). HRMS 395.0413, calcd for $C_{13}H_{17}O_9SNa_2$ 395.0389.

RESULTS AND DISCUSSIONS

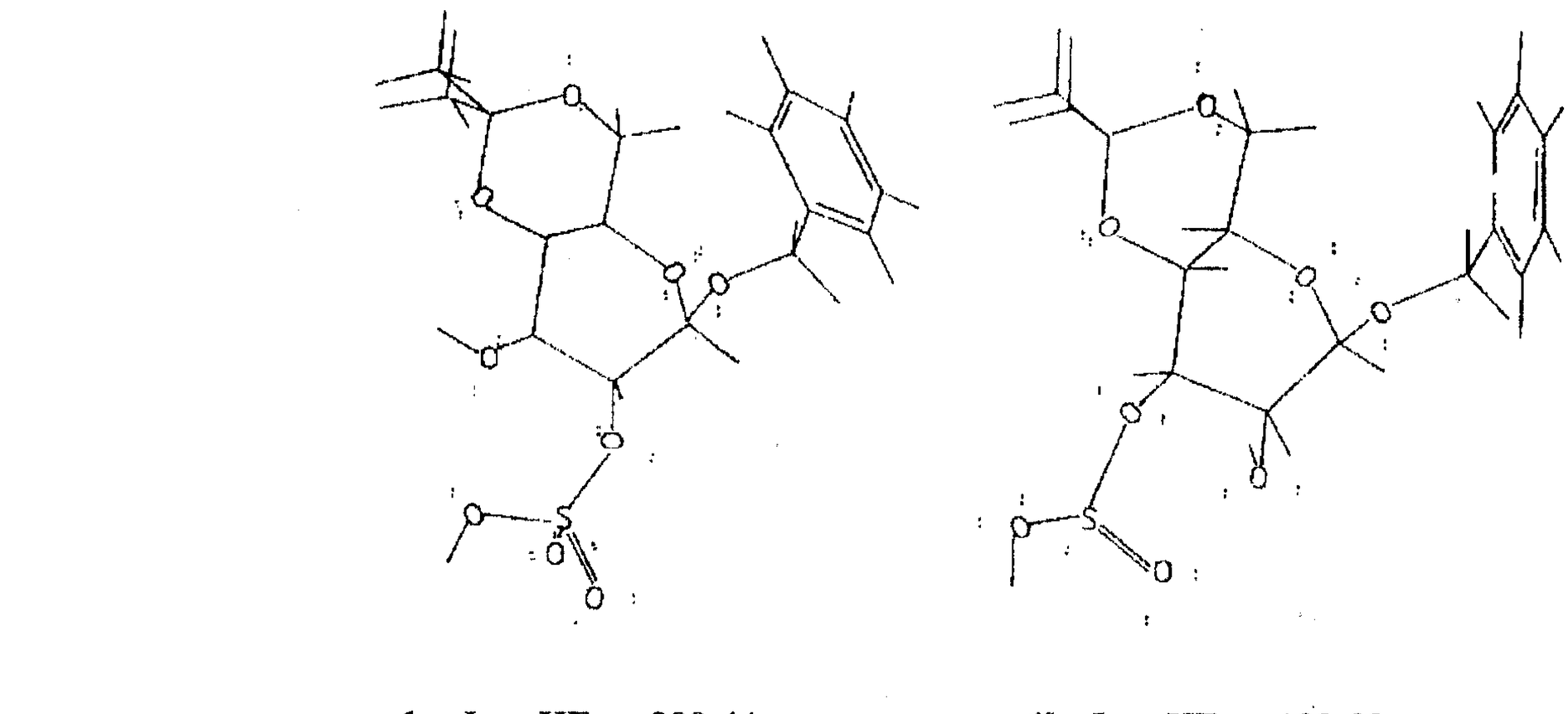
Glucose was first transformed into a mixture of benzyl glucosides **2** and **3** under Fischer glycosidation conditions by treatment with anhydrous benzyl alcohol and hydrochloric acid[4]. This mixture was used without purification in the formation of the cyclic 4,6-acetonides **4** and **5** with 2,2-dimethoxypropane, which could be separated on a preparative scale by MPLC.

Regioselectivity in sulfonylation of α- and ß-glucosides has been observed to follow the

reactivity order O-2 > O-4 > O-3 for α-glucosides, and O-4 > O-3 > O-2 for the ß-anomers[5]. It has also been observed that the regioselectivity of the ß-anomers is usually poorer than that of the corresponding α-anomers[6]. When the α-glucoside **4** was subjected to the sulfation conditions (pyridine-sulfur trioxide complex in pyridine)[7], the sulfated, acetal cleaved target compound **6** was obtained as the major product in a single step. The corresponding 3-sulfated isomer could not be detected by NMR or TLC. The high selectivity of 2- vs. 3-sulfation can be rationalized by the neighboring group participation of the 1-α-glycoside function, and the cleavage of the acetonide occurred during the reaction conditions due to its liability towards the developing acidic conditions [alternatively the acetonide can be cleaved by 80% aq. AcOH (1-2 hr, rt.) in a comparable yield].

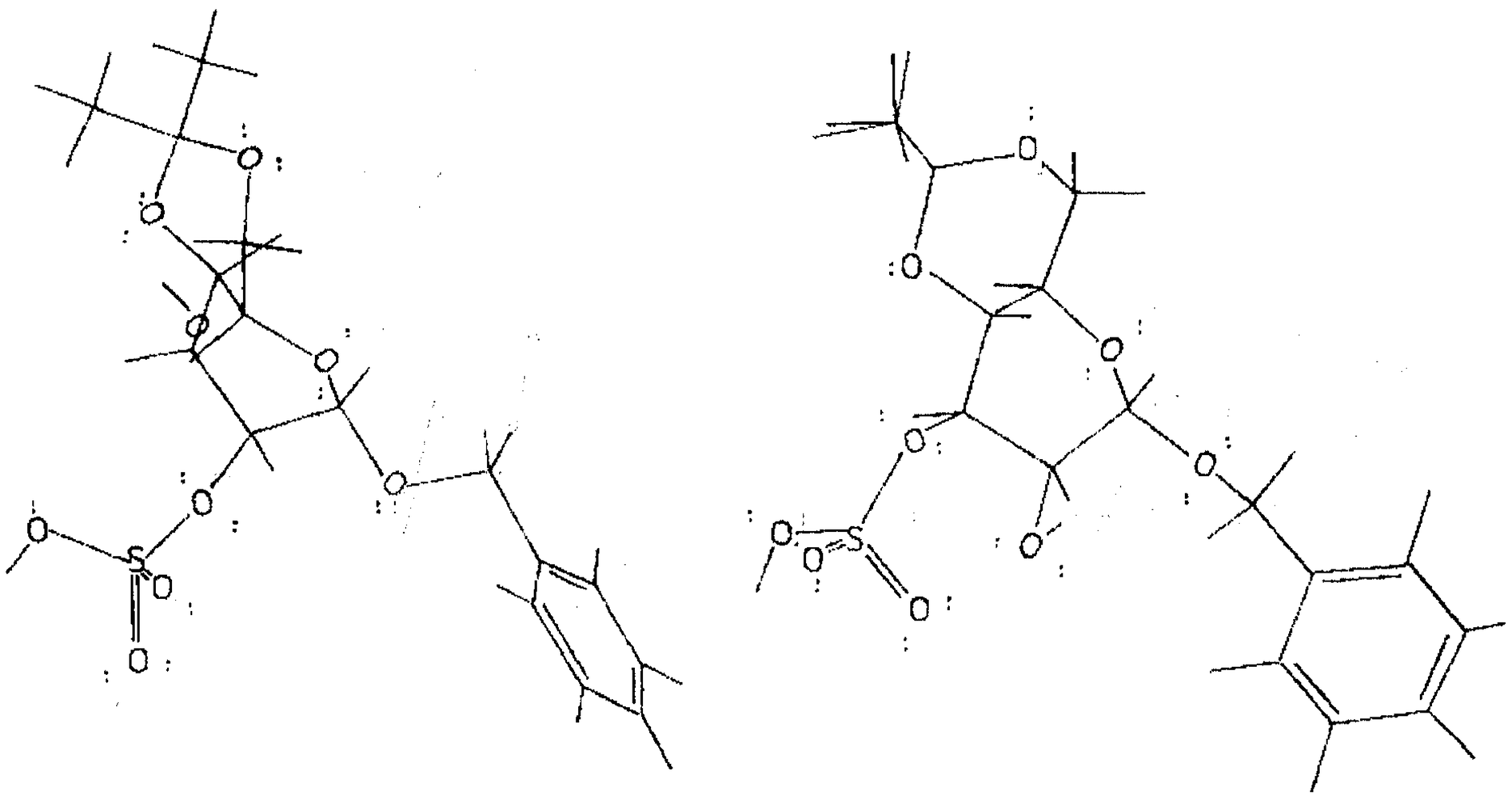
When the 1-ß-glucoside **5** was subjected to similar conditions, a mixture of two sulfated compounds **1** and **7** as free acids was obtained in approximately equal amounts. Again, the acetonide group had been cleaved, and the two compounds could be separated by preparative TLC. Comparison of the spectral data for the less mobile product with those reported for the authentic salvadoside[2] proved them to be identical. In the special techniques of chemical ionization FAB MS and HRMS, sodium metal was used in order to facilitate the detection of the molecular ion peaks of the compounds. Two sodium atoms were added to afford a new parent pak P= $[M+2Na]^+$ for the synthesized free acid of **1**, however, in case of the natural salvadoside which carries a sodium radical, it required one additional sodium atom. The same parent peak was obtained but identified as parent peak P= $[(M+Na)+Na]^+$, since the molecule is naturally found as a sodium salt. All of the daughter fragments appeared at the same molecular weights and intensities.

The fully optimized molecules to their minimal energy levels[8], or the most stable conformations agree with the reported selectivity for similar glycosides. In the case of acetonides of ß-D-glucopyranoside, the heat of formation of the 2-*O*-sulfate isomer was found HF= -200.44 (Fig. 1a) and that of the 3-*O*-isomer= -199.83 (Fig. 1b). This small difference did not favor the



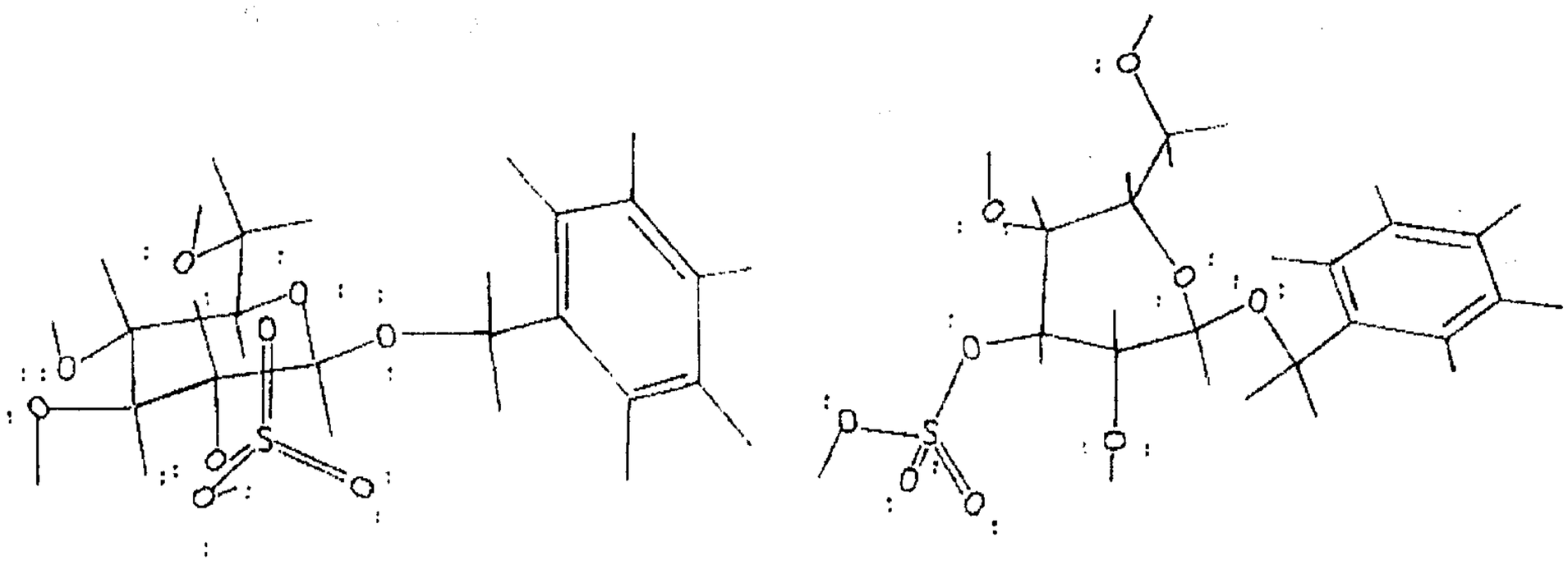
1a: Inc. HF= -200.44 **1b**: Inc. HF= -199.83

Fig. 1: Stereoscopic modeling of the acetonides of 1-*O*-benzyl-ß-D-glucopyranoside-sulfates (**1a** for 2-*O*-sulfate, **1b** for 3-*O*-sulfate).



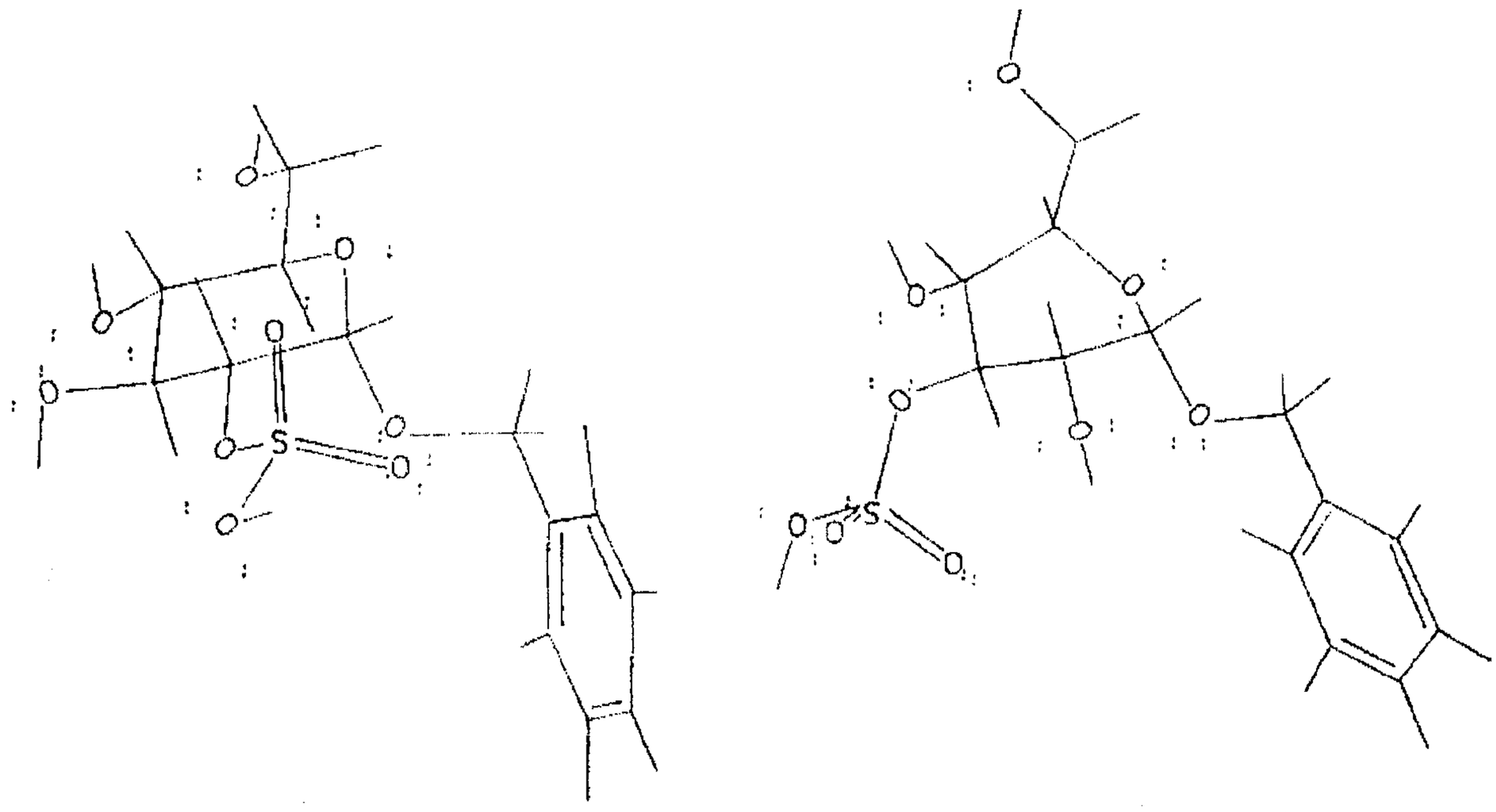
2a: Inc. HF= -200.48 **2b**: Inc. HF= -196.16

Fig. 2: Stereoscopic modeling of the acetonides of 1-*O*-benzyl-α-D-glucopyranoside-sulfates (**2a** for 2-*O*-sulfate, **2b** for 3-*O*-sulfate).



3a: Inc. HF= -207.88 **3b**: Inc. HF= -201.81

Fig. 3: Stereoscopic modeling of the final 1-*O*-benzyl-ß-D-glucopyranoside-sulfates (**3a** for 2-*O*-sulfate, **3b** for 3-*O*-sulfate).



4a: Inc. HF= -209.79 **4b**: Inc. HF= -200.79

Fig. 4: Stereoscopic modeling of the final of 1-*O*-benzyl-α-D-glucopyranoside-sulfates (**4a** for 2-*O*-sulfate, **4b** for 3-*O*-sulfate).

formation of 2-*O*- Vs 3-*O*-isomer and resulted in the proportions cited (experimental). The larger difference in HF of the acetonide of α-D-glucopyranoside of 2-*O*-sulfate (HF= -200.48, Fig. 2a) than that of the 3-*O*-isomer(HF= -196.16, Fig. 2b) favored the formation of only the 2-*O*-isomer and agreed with the NMR monitoring of the reaction.

On the other hand, the calculated HF of the final 1-*O*-benzyl-D-glucopyranoside-sulfates for all possible derivatives (Compounds **1,6,7**, Fig. 3a,b and Fig. 4a), in addition to the hypothetical compound (Fig. 4b) gave much more stable derivatives than their corresponding acetonide intermediates. These differences in the stability seems reasonable in the isolation of the final compounds rather than their acetonides during the reaction conditions and workup.

Acknowledgments

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