chromatography using SE-30 (T_R 10.3 and 6.2). The two components were isolated from this mixture by column chromatography on silica gel with benzene.

Digeranyl Ether——The component from the first eluate (*Rf* 0.42 and T_R 10.3) showed n_D^{25} 1.4829, d_4^{25} 0.8728, mol. wt., 290 (mass spectrum), 289 (Rast method). This compound in acetic acid absorbed 4 mole equivalents of hydrogen in the presence of platinum oxide.

Linalyl Geranyl Ether——The component from the second eluate, *Rf* 0.70 and T_R 6.2, showed n_D^{25} 1.4815, d_4^{25} 0.8805, mol. wt., 290 (mass spectrum), 288 (Rast method). This compound in acetic acid absorbed 4 mole equivalents of hydrogen in the presence of platinum oxide.

Monoterpene Hydrocarbon——The components of the fraction 1, bp 80—100° (10 mmHg), were detected by gas chromatography using carbowax 6000 at 80° and confirmed by addition of authentic specimens. Maleic anhydride was reacted with the fraction 1 for 20 days at room temperature. After the separated crystals were removed by filtration, the remaining oil was gas chromatographed. The peaks assigned to α-terpinene and myrcene disappeared, and the peak assigned to γ-terpinene and β-phellandrene (these showed the same retention value) was reduced.

Linalool——The compound which showed T_R 2.9 on gas chromatography with thermol-1 was isolated by distillation from the fraction 2, bp 100—110° (10 mmHg). The IR spectrum is identical with that of linalool.[4] Phenylurethane, mp 62.5—63.0°, $[\alpha]_D^{25} \pm 0$.

α-Terpineol and Geraniol——After elution by *n*-hexane to separate hydrocarbons, two components (T_R 4.5 and 7.0 on gas chromatography using thermol-1) were isolated by column chromatography on silica gel using ethyl acetate. The IR spectrum of the first eluate (T_R 4.5) is identical with that of α-terpineol.[5] Phenylurethane, mp 112.5—113.0°. The IR spectrum and phenylurethane, mp 123.5—124.0°, of the second eluate (T_R 7.0) are identical with those of geraniol.

Gas Chromatography——A Shimadzu GC-2 apparatus equipped with a thermal conductivity detector was used. For monoterpene hydrocarbon analysis, celite 545 (60—80 mesh) coated with carbowax 6000 (30%) was used at 80°, and for monoterpene alcohol, quartz powder (150—200 mesh) coated with thermol-1 (5%) was used at 100°. Carrier gas, helium; flow rate, 60 ml/min. For di-monoterpene ether, a Hitachi GCF-2 apparatus equipped with a flame ionization detector, and diasolid (60—80 mesh) coated silicon SE-30 (2%) was used at 200°.

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Symmetric Neutral Sulfates of Carbohydrates

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Symmetric neutral sulfates of diisopropylidene hexoses were synthesized from structural interest and their behavior to sodium methoxide was tentatively investigated.

Preparation of bis (1,2;5,6-di-O-isopropylidene-D-glucofuranose) 3,3′-sulfate (III) and bis-(1,2;3,4-di-O-isopropylidene-D-galactopyranose) 6,6′-sulfate (VII) was achieved by permanganate oxidation of their sulfite esters, (II) and (VI), respectively, which were obtained from the corresponding diisopropylidene hexoses, (I) and (V), respectively, by reaction of equivalent amount of thionyl chloride in the presence of pyridine. The reaction of thionyl chloride with 2,3:5,6-di-O-isopropylidene-D-mannofuranose (VIII), however, gave low yield of 2,3;5,6-di-

1) Location: 6-5 *Toneyama, Toyonaka, Osaka.*

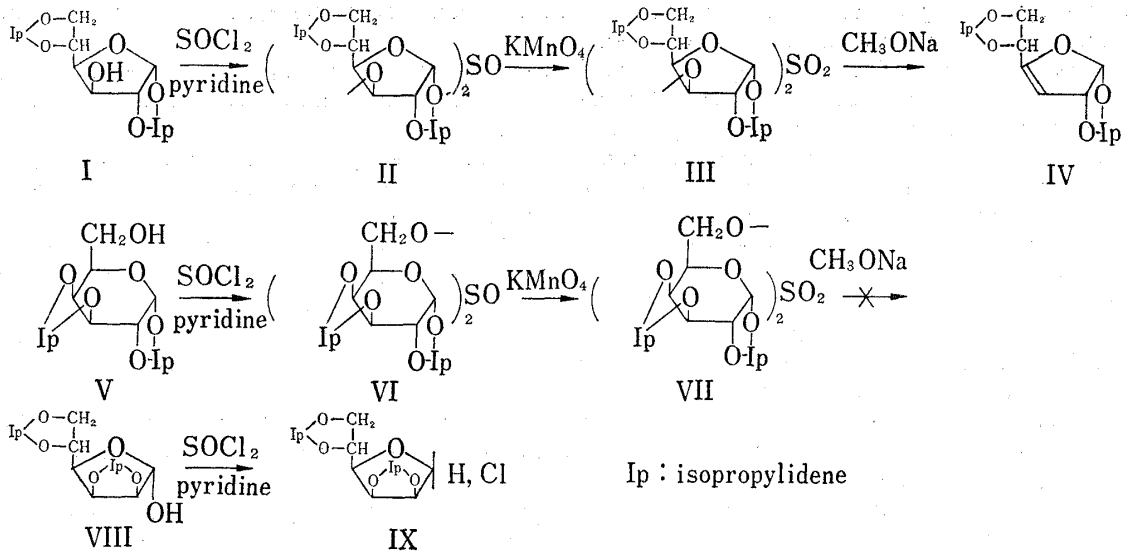

Chart 1

O-isopropylidene-D-mannofuranosyl chloride (IX), but no trace of sulfite ester. Thus, the sulfates of primary and secondary hydroxyl groups were obtained in good yield, while the attempt to prepare the sulfate of a hemiacetal hydroxyl group was unsuccessful.

The reaction of III with sodium methoxide gave, contrary to our expectation that it might give the methyl ether of diisopropylidene glucose like the reaction of dimethyl sulfate with sodium alkoxide, a small amount of 3-deoxy-1,2;5,6-di-O-isopropylidene-α-D-*erythro*-hex-3-enofuranose (IV); *i.e.*, desulfation leading to an unsaturated derivative preferred to the formation of 3-methylated derivative of I. With VII no reaction occurred even after heating at 130° in a sealed tube for 3 hours.

Experimental

Bis(1,2;5,6-di-O-isopropylidene-D-glucofuranose) 3,3′-Sulfite (II)——To 125 ml of an ethereal solution containing 13.0 g (5×10^{-2} mole) of 1,2;5,6-di-O-isopropylidene-D-glucofuranose (I) and 4.0 g (5×10^{-2} mole) of anhydrous pyridine was added dropwise during 30 min, with vigorous stirring and keeping the temperature below 0°, 25 ml of an ethereal solution containing 3.0 g (2.5×10^{-2} mole) of thionyl chloride. The reaction mixture was allowed to stand below 0° for an additional hour to complete the reaction. The precipitate of pyridine hydrochloride was filtered and the filtrate was evaporated to dryness. The residual syrup was dissolved in 100 ml of hexane and extracted thrice with 100 ml of water to remove the unreacted I. The hexane layer was dried with calcium chloride and evaporated to dryness to give 12.0 g (84.8%) of syrupy (II). $[\alpha]_D^{17}$ +0.3° (c=0.70, chloroform). *Anal.* Calcd. for $C_{24}H_{38}O_{13}S$: C, 50.87; H, 6.76; S, 5.66. Found: C, 51.25; H, 6.97; S, 5.67.

Bis(1,2;5,6-di-O-isopropylidene-D-glucofuranose) 3,3′-Sulfate (III)——To 100 ml of an acetic acid solution containing 12.0 g of II was added dropwise during 1 hr, with constant stirring, 100 ml of a 5% aqueous solution of potassium permanganate. Stirring was continued for an additional hour after the addition of potassium permanganate. The reaction mixture was extracted thrice with 100 ml of ether. The combined extracts were washed with 500 ml of a 5% aqueous solution of sodium bicarbonate and dried with calcium chloride. After evaporation of the solvent 9.2 g of syrupy crude (III) was obtained. The crude product was absorbed on 5 g of Wakogel C-200, placed on a Wakogel C-200 column (2.5×30 cm), and eluted with solvent system, benzene–ethyl acetate (7:3). From the fractions 4—6 (each fraction was 50 ml) 6.7 g (54%) of pure III was obtained, which was crystallized from methanol to give needles of mp 90°, $[\alpha]_D^{19}$ −53° (c=0.92, chloroform). *Anal.* Calcd. for $C_{24}H_{38}O_{14}S$: C, 49.48; H, 6.57; S, 5.51. Found: C, 49.24; H, 6.67; S, 5.19.

Bis(1,2;3,4-di-O-isopropylidene-D-galactopyranose) 6,6′-Sulfite (VI)——From 13.0 g of 1,2; 3,4-di-O-isopropylidene-D-galactopyranose (V) 13.7 g (96.8%) of syrupy (VI) was obtained in the similar manner as described for II. $[\alpha]_D^{17}$ −68° (c=0.70, chloroform). *Anal.* Calcd. for $C_{24}H_{38}O_{13}S$: C, 50.87; H, 6.76; S, 5.66. Found: C, 50.73; H, 6.77; S, 5.42.

Bis(1,2;3,4-di-O-isopropylidene-D-galactopyranose) 6,6′-Sulfate (VII)——From 13.5 g of VI 9.2 g of crude (VII) was obtained in the similar manner as described for III, which was purified on a Wakogel

C-200 column to give 5.9 g (42%) of pure VII. Crystallization from methanol gave prisms of mp 127°, $[\alpha]_D^{23}$ −66.5° (c=1.00, chloroform). *Anal.* Calcd. for $C_{24}H_{38}O_{14}S$: C, 49.48; H, 6.57; S, 5.51. Found: C, 49.39; H, 6.74; S, 5.45.

2,3;5,6-Di-O-isopropylidene-D-mannofuranosyl Chloride (IX)——To 12.5 ml of an ethereal solution containing 1.3 g (5×10^{-3} mole) of 2,3;5,6-di-O-isopropylidene-α-D-mannofuranose (VIII) and 0.40 g (5×10^{-3} mole) of anhydrous pyridine was added dropwise during 30 min, with vigorous stirring and keeping the temperature below 0°, 5 ml of an ethereal solution containing 0.30 g (2.5×10^{-3} mole) of thionyl chloride. The reaction mixture was allowed to stand below 0° for an additional hour, and the precipitate was filtered off. The filtrate was evaporated to dryness, absorbed on 2 g of Wakogel C-200, placed on a Wakogel C-200 column (1.5×45 cm), and eluted with the solvent system, hexane–ether (7:3). From the fractions 11—15 (each fraction was 10 ml) 58 mg (4.2%) of a syrup was obtained, which gave a single spot on TLC. $[\alpha]_D^{25}$ +17° (c=0.89, chloroform) (lit.[2]: $[\alpha]_D^{21}$ of the α-anomer, +85.7°). *Anal.* Calcd. for $C_{12}H_{19}O_5Cl$: C, 51.73; H, 6.87; Cl, 12.70. Found: C, 51.73; H, 7.28; Cl, 12.67.

3-Deoxy-1,2;5,6-di-O-isopropylidene-α-D-*erythro*-hex-3-enofuranose (IV)——The mixture of an *o*-xylene solution containing 346 mg of (III) and 5 ml of 1N methanolic sodium methoxide was heated at 100° for 10 min to evaporate methanol. Then the remaining solution was refluxed for 3 hr. The reaction mixture was evaporated to dryness and the residue was extracted twice with 10 ml of ether. After evaporation of the solvent 156 mg of a dark brown syrup was obtained. Thin-layer chromatography (TLC) of the syrup showed a fast moving spot (major) along with two slower moving spots (minor). The major component was isolated by Wakogel C-200 column (0.5×30 cm) chromatography to give 78 mg (54%) of a syrup, which was crystallized from ether to give needles of mp 50.5° (lit.,[3] 51°), $[\alpha]_D^{21}$ +19.7° (c=0.61, chloroform) (lit.[3]: $[\alpha]_D^{20}$ +19.8°, c=3.0, ethanol). *Anal.* Calcd. for $C_{12}H_{18}O_5$: C, 59.45; H, 7.50. Found: C, 59.51; H, 7.42.

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Biochemical Syntheses. VIII.[1] Microbial Transformation of α-Santonin to 1,2-Dihydro-α-santonin[2]

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In the recent few years we have performed microbial transformation of a number of sesquiterpenoids obtained from plants and found that some biological procedures (*i.e.* selective hydroxylation) which actually take place in the plants can be reproduced by enzymes induced by microorganisms.[4–6]

α-Santonin (I) and artemisin (II) are the sesquiterpenic constituents of *Artemisia* spp. (Compositae) and the latter must be biosynthesized by enzymatic hydroxylation of the former in the plants.

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