A NEW MICROBIAL METABOLITE, SPHYDROFURAN. II

THE STRUCTURE OF SPHYDROFURAN

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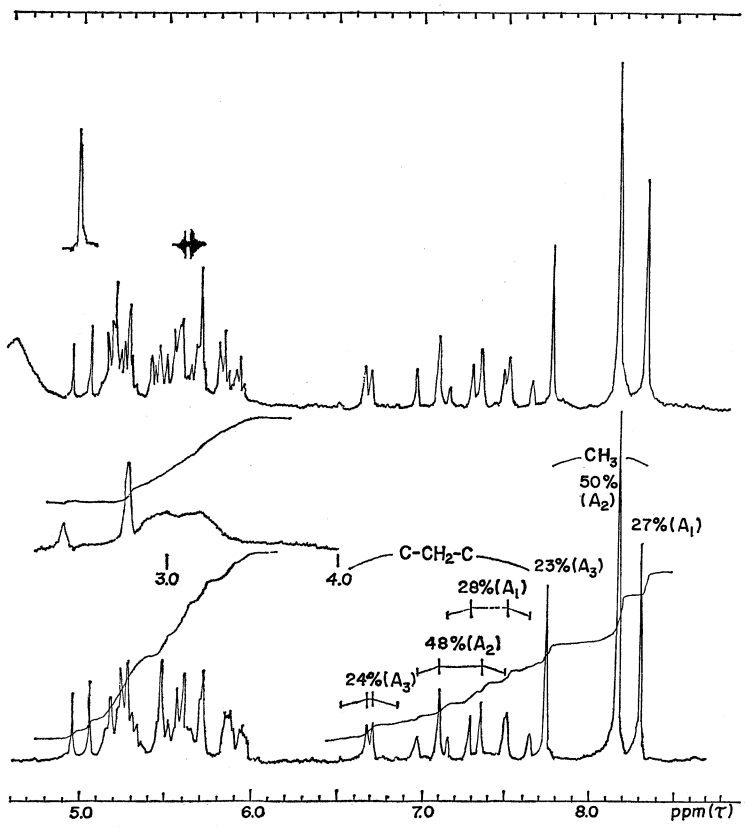
The structure of a new streptomycete metabolite sphydrofuran was determined as an anomeric mixture of 3,4,5′-trihydroxy-5′-methyl-2,3′-spirobi (tetrahydrofuran) (A_1 and A_2) which is brought into an equilibrium mixture with *trans*-3, *cis*-4-dihydroxy-*trans*-2-hydroxymethyl-2-acetonyl(tetrahydrofuran) (A_3) in some solvents.

In the previous paper[1] the isolation of sphydrofuran and the structure of a hydrolysis product was described. This paper describes the structure of sphydrofuran.

The NMR spectrum of sphydrofuran (Fig. 1) distinctly showed three singlets which were considered to originate from methyl protons (τ 8.30, 8.16 and 7.74). However, it was found that the strength and the shift-values of these signals were influenced by changing the solvent (see Figs. 1, 2 and 3). Originally, the sample of sphydrofuran was suspected to be impure,

Fig. 1. NMR spectrum of sphydrofuran in pyridine-d_5 with (upper) and without (lower) D_2O at 100 MHz.



however, repeated chromatography and recrystallization did not change the melting point (99.5~101°C) and the optical rotation ($[\alpha]_D$ +18° in water). Consequently, sphydrofuran was considered to be an equilibrium mixture of three components and the high yield (83 %) of transformation[1] of sphydrofuran to its hydrolysis product (I) in dilute acid without formation of any other product also supported this possibility. Further inspection of the NMR spectrum (Fig. 1) of sphydrofuran in pyridine-d_5 also

Fig. 2. NMR spectrum of sphydrofuran in D_2O at 100 MHz.

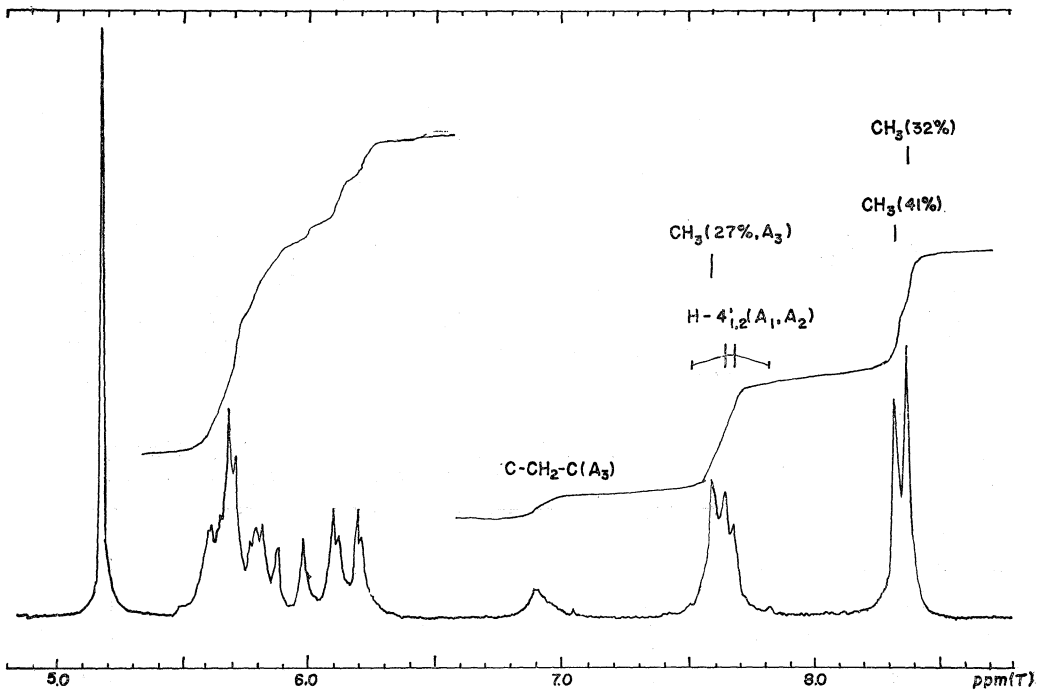


Fig. 3. NMR spectrum of sphydrofuran in DMSO-d_6 at 100 MHz.

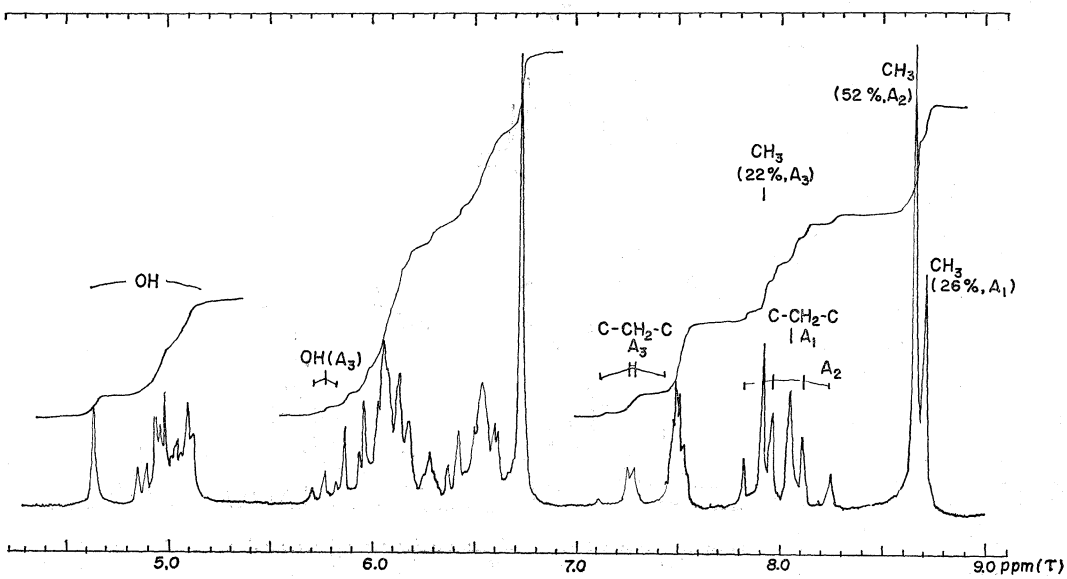
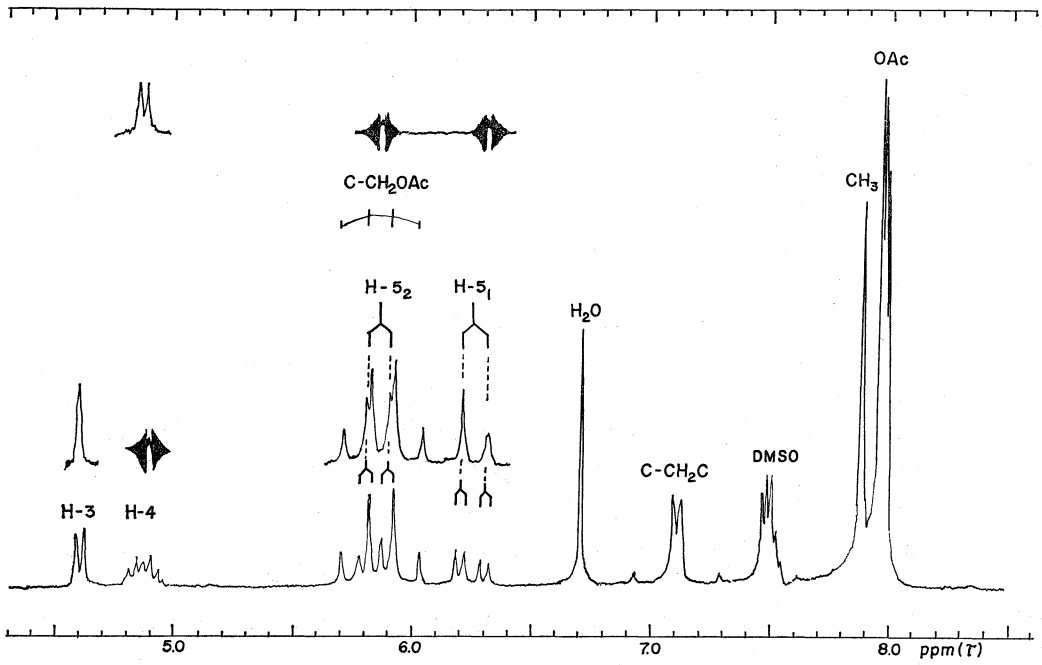


Fig. 4. NMR spectrum of triacetate (II) of sphydrofuran in DMSO-d_6 at 100 MHz.



supported the possibility. In the spectrum, the signals ranging τ 5~6 were too complicated for analysis, whereas the signals ranging τ 6.5~8 were better separated and easier to analyze.

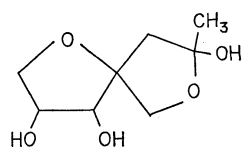
The proportion of the amounts of possible equilibrium isomers were estimated from the integrations of the three above-mentioned methyl signals at τ 8.30, 8.16 and 7.74 and the values obtained were 27, 50 and 23 %, respectively. On the other hand, signals ranging τ 6.5~7.6 also could be separated into three groups of AB quartets as shown in Fig. 1 and the proportion of the integration of the quartets were again 28, 48 and 24 % in the order of the quartets at τ 7.39, 7.23 and 6.69. Each one of the quartets could be assigned to an isolated methylene protons from their coupling constants (J_{AB}~14 Hz) and the double irradiation results. The accordance of the two proportion values thus confirmed the presence of three structurally related isomers and they were tentatively named as components A_1, A_2 and A_3 corresponding to the methyl signals at τ 8.30, 8.16 and 7.74.

In the next place, sphydrofuran was acetylated in pyridine with acetic anhydride to give a chromatographically homogeneous syrup ($[\alpha]_D^{20}$ −18° in methanol) in a yield of 71 %. The mass spectrum of the acetylated product (II) showed the molecular ion at m/e 316 and the value was in accordance with the formula ($C_{14}H_{20}O_8$) estimated from the elemental analysis of II. Since the experimental formula of sphydrofuran is $C_8H_{14}O_5$[1], the above formula should be $C_{14}H_{20}O_8 = C_8H_{14}O_5 + 3CH_2CO$ corresponding to the formula of a triacetate of sphydrofuran. It was noteworthy that the acetylated product (II) was spectrometrically homogeneous and not a mixture of several components, as is the case for sphydrofuran. Moreover, the NMR spectrum (Fig. 4) of II showed that the shift-value (τ 7.88) of the methyl and the pattern of the

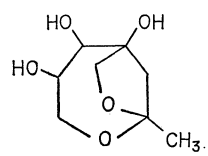
methylene protons (τ 7.11) were similar to those of the component A_3. Each signal group of II was assigned as shown in Fig. 4 mainly by double resonance techniques (see experimental), and the presence of a sequence of -CH(OAc)CH(OAc)CH_2O- was revealed. The same sequence was indicated in the tri-O-acetylated product[1] of the hydrolysis product (I) of sphydrofuran.

When II was deacetylated in methanol with a catalytic amount of sodium methylate to obtain a deacetylated product, the product obtained was identical with sphydrofuran, the NMR spectrum was the same as that of authentic sphydrofuran which is a mixture of three components.

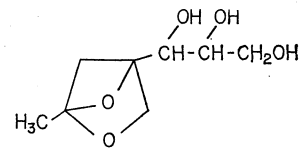
The recovery of sphydrofuran from II indicates the presence of a sequence of -CH(OH)CH(OH)CH_2O- in sphydrofuran itself, and the transformation of sphydrofuran into its hydrolysis product (I) in mild acidic condition suggests that the molecular framework of sphydrofuran is the same as that of the hydrolysis product. The IR[1] and NMR spectra show that sphydrofuran has no carbonyl in solid state (from IR) or unsaturated (from NMR) groups. In view of the presence of three hydroxyl groups and two ether linkages, the experimental formula ($C_8H_{14}O_5$) of sphydrofuran indicates that it is a bicyclic compound. On the basis of the spectroscopic and chemical evidence described above, five possible structures (A, B, C, D and E) were proposed for sphydofuran:



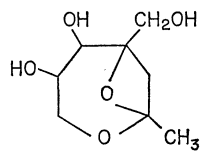
(A)



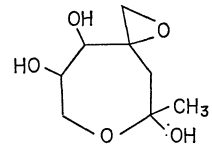
(B)



(C)



(D)



(E)

The NMR spectra of sphydrofuran and its tri-O-acetylated derivative (II) can be explained by any of these formulae. However, structure B is not reasonable because generally, tertiary hydroxyl group can not be easily acetylated.

During hydrolysis of sphydrofuran in acidic methanol, a pair of short-life intermediates were recognized on TLC, and on further hydrolysis, these intermediates gave the hydrolysis product (I) which is obtained in aqueous acidic solution. Furthermore, these intermediates could be restored to the starting material (sphydrofuran) on addition of water to the reaction mixture. These intermediates were successfully isolated by the following procedure: A solution of sphydrofuran in 0.001 N hydrogen chloride in methanol was allowed to stand at room temperature (22°C) for 30 minutes, whereupon the starting material had disappeared and a pair of EHRLICH-positive products were formed (on TLC, Rf 0.47 (III_a, major) and 0.44 (III_b,

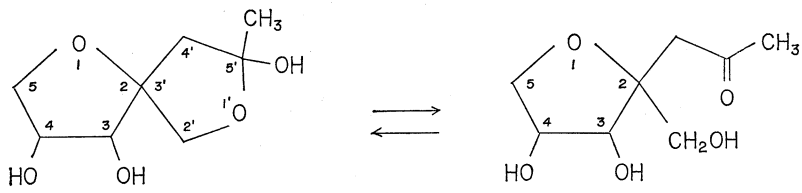
minor)). At this stage, when water was added to the reaction mixture, III_a and III_b were transformed into the starting material within several minutes. This fact indicates that III_a and III_b still maintain the basic structure of sphydrofuran. If the reaction mixture was neutralized with basic resin, the solution, after evaporation, gave a syrup, which was chromatographed on a column of Dowex 1×4 (OH form) to give III_a and III_b in yields of 54 and 13% respectively; III_a, m.p. 95~100°C, $[\alpha]_D^{21}$ −56° (in methanol) and III_b, syrup, $[\alpha]_D^{25}$ +130° (in methanol).

The NMR spectra (Figs. 5 and 6) of III_a and III_b were similar and indicated the presence of a methyl (singlet each, τ 8.46 and 8.54 in the order of III_a and III_b), an O-methyl (τ 6.77 and 6.65), an isolated methylene O-CH_2-C (each AB quartet centered at 5.49 and 5.43), and a grouping of -CH(OH)CH(OH)CH_2O- (each in the range of τ 5.2~6).

The elemental analysis of III_a and III_b also showed that both compounds were mono-O-methylated derivatives of sphydrofuran. The formation of these O-methylated compounds from sphydrofuran in anhydrous alcoholic solution of hydrogen chloride and the remarkable difference in optical rotations (186°) between III_a and III_b suggested that the nature of the O-methyl groups in III_a and III_b resembles those of cyclic hemiketal groups in carbohydrates. Furthermore, acetylation of a mixture of III_a and III_b with acetic anhydride in pyridine gave a mixture of di-O-acetylated products (IV-Mixture), and its NMR spectrum showed the presence of the grouping of -CH(OAc)CH(OAc)CH_2O-. On the basis of these results, the above mentioned structures B, C and D should be excluded.

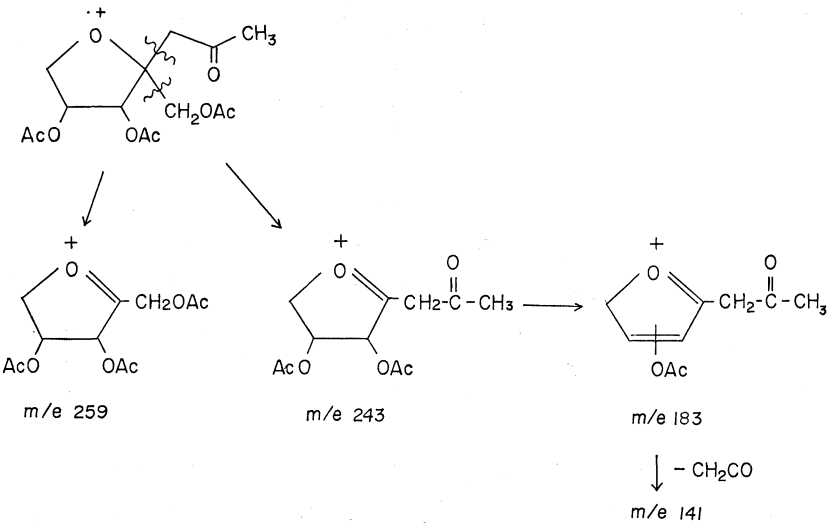
The chemical shifts of the isolated methylene protons (O-CH_2-C, τ 5~6) in sphydrofuran, II, III_a or III_b (see experimental) are much smaller than the chemical shift of the methylene group in an oxirane ring (τ 6.8~7.9[2]); this may exclude the formula E. Furthermore, the reduction products (V-Mixture) of sphydrofuran, which will be described later, were found to be stable in an acidic medium in which generally an oxirane ring is unstable[3].

From the results and discussion described above, it may be concluded that the sphydrofuran is an anomeric mixture of 3,4,5′-trihydroxy-5′-methyl-2,3′-spirobi(tetrahydrofuran) (A_1 and A_2) which is brought into an equilibrium mixture with 2-acetonyl-3,4-dihydroxy-2-hydroxymethyltetrahydrofuran (A_3) in some solvents.



Anomers (A_1, A_2) A_3

The structure of A_3 was supported from the results of the mass spectrum of II (m/e 316, 259, 243, 196, 183, 141, 99). The fragmentation pattern was interpreted as follows:



In addition, the structures A_1, A_2, A_3 were further confirmed as follows: when sphydrofuran was hydrogenated over a palladium catalyst, no reaction occurred, however, on treatment with sodium borohydride, a mixture of a pair of derivatives (**V**-Mixture) was obtained. This mixture was proved to be acid-stable but gave a negative reaction with EHRLICH reagent. The NMR spectrum of **V**-Mixture showed a pair of doublets (J 6 Hz) in the ratio of approximately 2.5 : 1 at τ~8.9 (methyl groups), and this suggested that the reduction occurred at the carbon next to the methyl group in sphydrofuran forming a methine and a hydroxyl group; the presence of two sets of four hydroxyl groups in **V**-Mixture was also shown from its O-acetylation products (**VI**-Mixture).

The presence of a pair of primary hydroxyl groups in **V**-Mixture was also indicated from the tritylation products (**VII**$_a$ and **VII**$_b$) of **VI**-Mixture. The products were separated, though in low efficiency, into a pair of mono-O-tritylated products, **VII**$_a$: $[\alpha]_D^{25}$ −4° (c 1, methanol), **VII**$_b$: $[\alpha]_D^{25}$ −14° (c 1, methanol). The easy mono-O-tritylation indicated the presence of a primary hydroxyl group[4].

V-Mixture showed an Rf 0.70 on TLC with silica gel and n-propanol - ethyl acetate - water (7 : 1 : 2), whereas in the presence of boric acid **V**-Mixture showed an Rf 0.52 under the same conditions. The NMR spectrum of **V**-Mixture in methanol-d_4 in the presence of boric acid was also different from that of **V**-Mixture in methanol-d_4 alone. These observations attributed to complex formation of **V**-Mixture and boric acid.

When **V**-Mitxture was treated with acetone in the presence of anhydrous cupric sulfate, a pair of acetonation products were formed and, contrary to the case of **VI**-Mixture, the two acetonation products were successfully separated by column-chromatography on silica gel eluted with benzene - ethanol (8 : 1) containing 0.1% triethylamine. The major product (**VIII**$_a$, Rf 0.29 with the same solvent system) had m.p. 74.5~75.5°C and $[\alpha]_D^{27}$ +32° (c 1, pyridine) and the minor (**VIII**$_b$, Rf 0.33), m.p. 121~122°C and $[\alpha]_D^{27}$ −25° (c 1, pyridine).

The acetonation products (**VIII**$_a$ and **VIII**$_b$) were then acetylated with acetic

Fig. 5. NMR spectrum of III_a in pyridine-d_5 at 100 MHz.

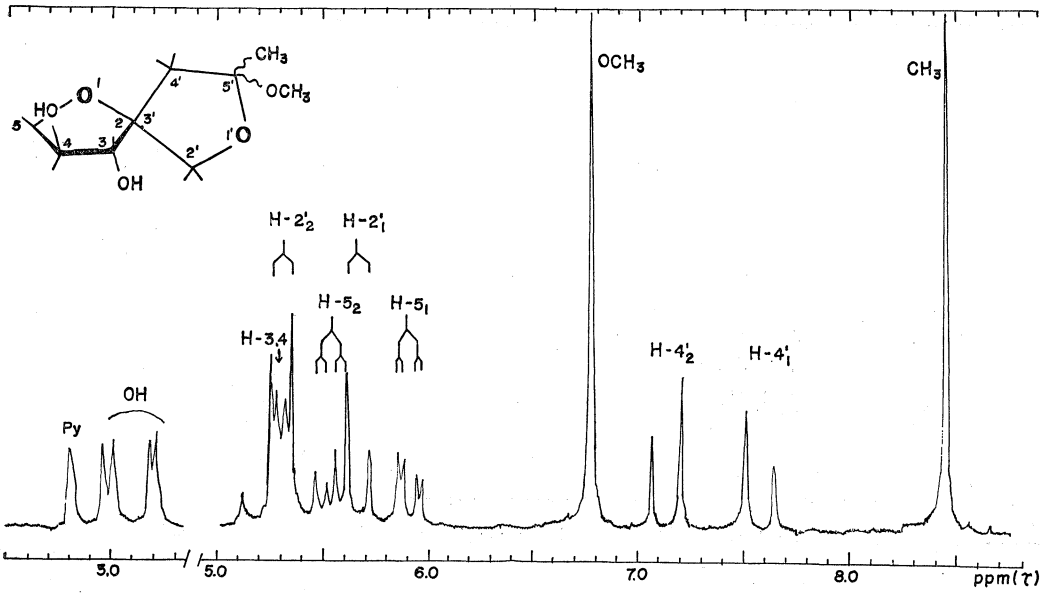
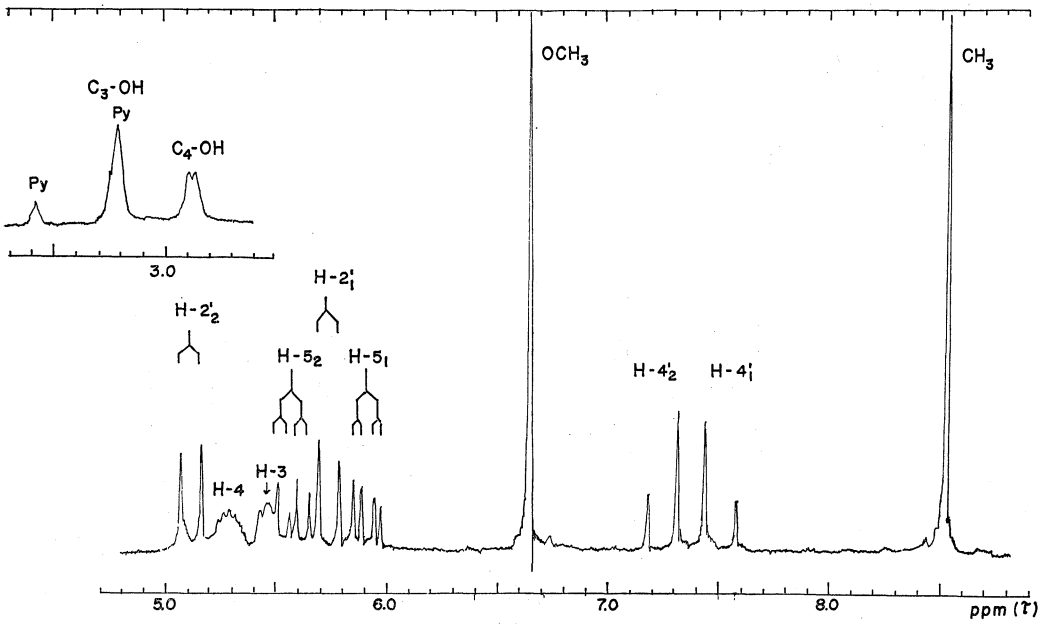


Fig. 6. NMR spectrum of III_b in pyridine-d_5 at 100 MHz.



anhydride in pyridine and diacetylated products (IX_a and IX_b) were obtained; IX_a: $[\alpha]_D^{27}$ +19° (*c* 1, benzene) and IX_b: $[\alpha]_D^{27}$ −41° (*c* 1, benzene). The NMR data of $VIII_a$, $VIII_b$, IX_a and IX_b are described in the experimental part and the spectra of $VIII_b$ and IX_b are shown in Figs. 7 and 8, respectively.

Comparison of the NMR data of $VIII_{a,b}$ and $IX_{a,b}$ indicated that the signals due to two methine protones of $VIII_{a,b}$ at τ∼5.35 (H-4*) and ∼5.5 (H-2′′*) shifted, on

* See the numbering of compounds $VIII_{a,b}$ in Chart 1.

Fig. 7. NMR spectrum of $VIII_b$ in pyridine-d_5 at 100 MHz.

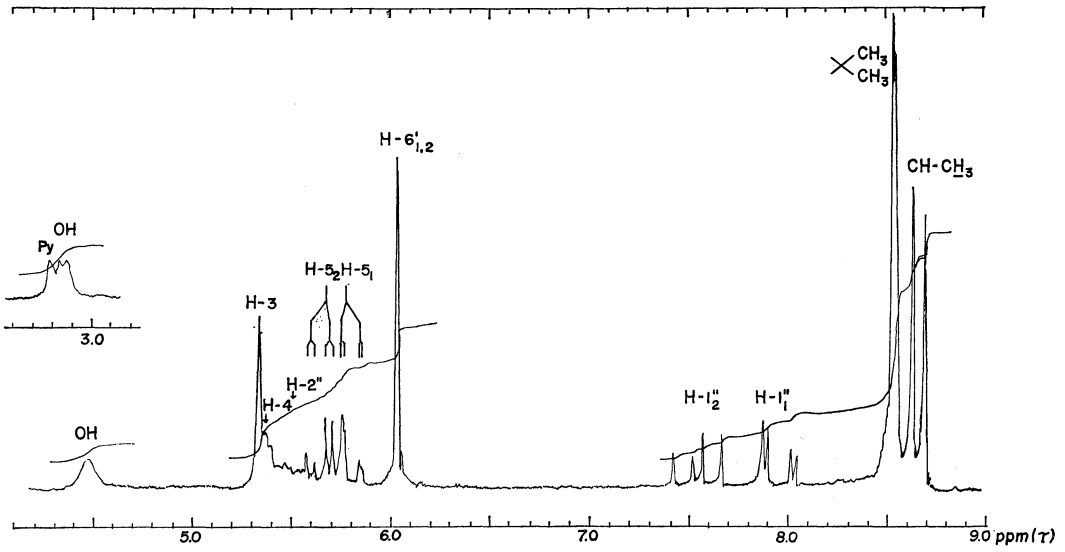
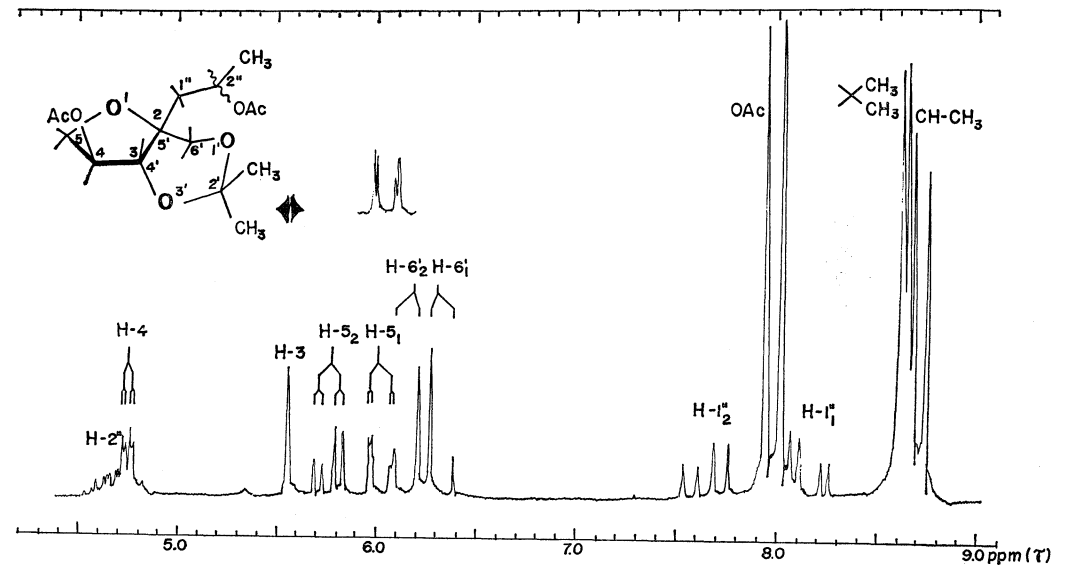


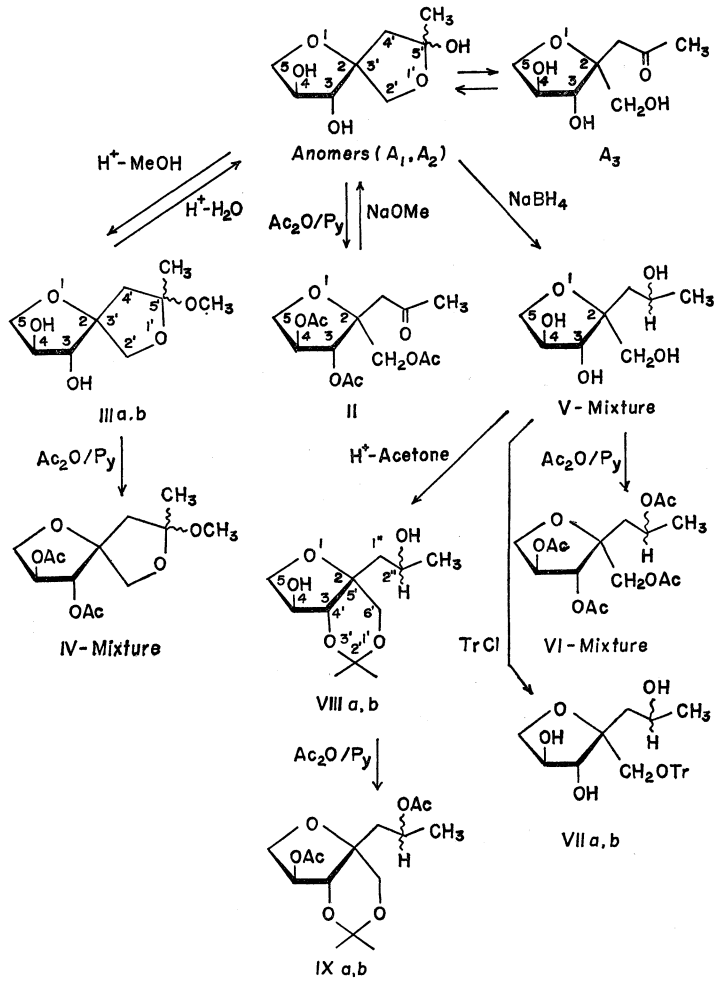
Fig. 8. NMR spectrum of IX_b in pyridine-d_5 at 100 MHz.



acetylation to give $IX_{a,b}$, downfield (τ~4.74 and ~4.65, respectively) and this suggested that the hydroxyl groups at C-4 and C-2″ in $VIII_{a,b}$ were acetylated and therefore, the hydroxyl group at C-3 and the primary hydroxyl group were acetonated to give a six-membered ring.

Inspection of molecular models of $VIII_{a,b}$ and $IX_{a,b}$ showed that such a ring could be formed only when the hydroxymethyl and the hydroxyl (C-3) groups are *cis*. The failure of acetonation between the hydroxyl groups at C-3 and C-4 led to the conclusion that the hydroxyl groups should be *trans*, because, in a 5-membered ring, ring closure of a 1,3-dioxacyclopentane is hardly possible. This conclusion was also

Chart 1.



supported from the NMR data of $VIII_{a,b}$ and $IX_{a,b}$; the coupling constant $J_{3,4}$ was ~0 Hz, indicating H-3 and H-4 are *trans*. Further support for these stereostructures was obtained by the nuclear OVERHAUSER effect in relation to IX_b. Irradiation of one of methyls (τ 8.65) of the isopropilidene group caused an increase of 18 % in the area of H-3 signal, but no increase in the H-$6_1'$ or H-$6_2'$ signals; this indicates the spatial proximity of the methyl and H-3 proton in IX_b and also suggests the skew-boat and not chair structure of the dioxacyclohexane ring. This finding also excludes the possibility, from the inspection of the molecular model, that the hydroxymethyl and the hydroxyl (C-3) groups are *trans*.

From the chemical and spectral information described above, the chemical structure of sphydrofuran is assigned as an anomeric mixture of a substituted 2,3'-spiro(tetrahydrofuran) stereochemically shown by the formula A_1 and A_2 in Chart 1 or by their enantiomers, and it has been shown that one of the tetrahydrofuran rings contains a lactole linkage which is capable of undergoing anomeric interconversion through a keto-form (formula A_3) isolated as an acetyl derivative (II). It is noteworthy that

the screening procedure with the EHRLICH reagent disclosed the presence of a spirotetrahydrofuran compound among microbial metabolites.

Experimental

The procedures for thin-layer chromatography and NMR spectra were the same as described in the previous paper[1].

NMR spectra of sphydrofuran at 100 MHz

(a) In pyridine-d_5. τ: 8.30 (27 %), 8.16 (50 %) and 7.74 (23 %) (three singlets, the integration of the three signals corresponded to three protons; each CH_3); 7.54 and 7.25 (each doublet, forming an AB quartet centered at 7.39, $J_{A,B}$ 13.5 Hz, H-$4_1'$, $4_2'$ in A_1), 7.40 and 7.06 (an AB quartet centered at 7.23 as described above, $J_{A,B}$ 13.5 Hz, H-$4_1'$, $4_2'$ in A_2), 6.73 and 6.65 (an AB quartet centered at 6.69 as described above, $J_{A,B}$ 14.5 Hz, $CH_3COC\underline{H}_2$ in component A_3). The relative proportion of the integrations of the three quartets was 28, 48 and 24 %, in the order as cited above and the total integration of the three quartets corresponded to two protons. τ 6.0~4.95 (6H, H-3, 4, 5_1, 5_2, $2_1'$, $2_2'$). On irradiation at τ 7.54 a doublet at τ 7.25 collapsed to a singlet; and on irradiation at τ 7.40 a doublet at τ 7.06 collapsed to a singlet.

(b) In pyridine-d_5 containing a small amount of deuterium oxide. τ: 8.32, 8.16 and 7.76 (three singlets, CH_3), 7.39, 7.22 and 6.67 (each AB quartet), 6.0~4.95 (6H m, the pattern was better resolved than that obtained in pyridine-d_5). On irradiation at τ 5.00, a singlet appeared at τ 5.62 and on irradiation at τ 5.62, a doublet at τ 5.00 collapsed to a singlet. As the integration of the doublet at τ 5.00 was about 0.5 proton, the above-mentioned AB quartet (?) (τ 5.00 and 5.62) may be assigned to a methylene at C-2' in component A_2.

(c) In deuterium oxide. τ: 8.37 (32 %), 8.33 (41 %) and 7.59 (27 %) (three singlets, each CH_3); 7.70 and 7.63 (each doublet, forming an AB quartet in total, J 14.5 Hz, -C-CH_2-C-); ~6.90 ($CH_3COC\underline{H}_2$ in A_3); 6.25~5.45 (6H, H-3, 4, 5_1, 5_2, $2_1'$, $2_2'$).

(d) In dimethylsulfoxide-d_6. τ: 8.70 (26 %), 8.66 (52 %) and 7.91 (22 %) (three singlets, each CH_3); 8.14 and 7.91 (each doublet, forming an AB quartet centered at τ 8.02, $J_{A,B}$ 13.8 Hz, H-$4_1'$, $4_2'$ in A_2), 8.04 (apparently singlet, H-$4_1'$, $4_2'$ in A_1), 7.30 and 7.24 (each doublet, forming an AB quartet centered at τ 7.27, $J_{A,B}$ 14 Hz, $CH_3COC\underline{H}_2$ in component A_3), 6.65~5.85 (6H, H-3, 4, 5_1, 5_2, $2_1'$, $2_2'$), 5.85~4.63 (3H, disappeared on deuteration, OH), 5.77 (triplet, disappeared on deuteration, $CH_2O\underline{H}$ in A_3). Irradiation at τ 6.55, a triplet at τ 5.77 collapsed to a singlet.

3,4-Diacetoxy-2-acetoxymethyl-2-acetonyltetrahydrofuran (II): To an ice cold solution of sphydrofuran (211 mg) in pyridine (5 ml), a cold mixture of acetic anhydride - pyridine (1 : 1, 1.5 ml) was added and the solution was allowed to stand overnight. After addition of a small amount of water, the solution was concentrated to a small volume, to which chloroform (30 ml) was added and the organic layer was washed successively with water, saturated potassium bisulfate solution, water, saturated bicarbonate solution and again with water, dried over sodium sulfate and evaporated. The resulting syrup was chromatographed on a column of silica gel (Mallinckrodt CC7, 20 g) with benzene - ether (2 : 1) and the fraction (60~145 ml) was evaporated. Addition of petroleum ether to the residue gave a syrup, 250 mg (71 %), $[\alpha]_D^{20}$ −18° (c 1, methanol), Rf 0.30 (with benzene - ether 2 : 1). Anal. Found: C 53.19, H 6.43. Calcd. for $C_{14}H_{20}O_8$: C 53.16, H 6.37 %.

IR spectrum (KBr disk): 1750, 1725 (shoulder), 1370, 1230, 1050, 900 cm^{-1}. Mass spectrum (m/e): 316 (molecular ion M^+), 259 ($M^+ - CH_3COCH_2$), 243 ($M^+ - CH_3CO_2CH_2$), 196, 183, 141, 99.

NMR spectrum at 100 MHz in dimethylsulfoxide-d_6: τ: 7.99, 7.97 and 7.96 (each 3H s, OAc), 7.88 (3H s, C-$COCH_3$), 7.14 and 7.08 (2H AB quartet centered at 7.11, $J_{A,B}$ 16 Hz, $CH_3COC\underline{H}_2$); 6.24 (1H q, H-5_1), 5.87 (1H q, H-5_2); 5.95 and 5.79 (2H AB quartet, $J_{A,B}$ 11.2 Hz, $AcOC\underline{H}_2$-C); 4.88 (1H sextet, H-4), 4.61 (1H d, H-3). $J_{3,4}$ 3.0 Hz, $J_{4,5_1}$ 3.7 Hz,

$J_{4,5_2}$ ~5.5 Hz, $J_{5_1,5_2}$ 10.2 Hz. Irradiation at τ 4.88 (H-4), two quartets at τ 6.24 (H-5_1) and 5.87 (H-5_2) collapsed to two doublets (J 10.2 Hz) and a doublet at τ 4.61 collapsed to a singlet. Simultaneous irradiation at τ 6.26 and 5.85, a sextet at τ 4.88 collapsed to a doublet (J 3.0 Hz).

5'-Anomers of 3,4-Dihydroxy-5'-methoxy-5'-methyl-2,3'-spirobi(tetrahydrofuran) (**III**$_a$ and **III**$_b$): A solution of sphydrofuran (357 mg) in 0.001 N hydrogen chloride in methanol (2 ml) was allowed to stand at room temperature for 30 minutes. On TLC (chloroform-methanol 7:1), sphydrofuran (Rf 0.23) disappeared and a pair of products (**III**$_a$, Rf 0.47 (major) and **III**$_b$, Rf 0.44 (minor)) appeared. Both were active for the EHRLICH reagent. The solution was passed through a column of Dowex 1×2 (OH form, 7 ml) which was pretreated with methanol. A fraction (4~13 ml) was evaporated to give a syrup (371 mg) which partially crystallized on standing. The product was then chromatographed with methanol on a column of Dowex 1×4 (OH form, 200~400 mesh, 2.1×80 cm) which was pretreated with methanol. A fraction (190~220 ml) was evaporated to give a syrup which crystallized on standing (**III**$_a$, 206 mg, 54 %). The product was recrystallized from chloroform-petroleum ether; m.p. 95~100°C, $[\alpha]_D^{21}$ −56° (c 1, methanol). From fraction (220~230 ml), a mixture of **III**$_a$ and **III**$_b$ was obtained (~100 mg). From another fraction (230~245 ml), a minor product **III**$_b$ was obtained as a syrup, 48 mg (13 %), $[\alpha]_D^{25}$ +130° (c 1, methanol). The IR spectra of both compounds were similar.

Anal. Found: **III**$_a$: C 53.07, H 7.83; **III**$_b$: C 52.73, H 8.19.
Calcd. for $C_9H_{16}O_5$: C 52.93, H 7.90 %.

NMR spectrum for **III**$_a$ at 100 MHz in pyridine-d_5. τ: 8.46 (3H s, C-CH_3), 7.55 and 7.15 (each 1H doublet, forming an AB quartet centered at τ 7.35, $J_{A,B}$ 13.8 Hz, H-$4_1'$, $4_2'$), 6.77 (3H s, OCH_3); 5.90 (1H q, H-5_1); 5.55 (1H q, H-5_2); 5.65 (1H d, H-$2_1'$), 5.32 (1H d, H-$2_2'$); ~5.30 (2H short-range peaks, H-3, 4); 3.21 (1H d, J 3.2 Hz, OH; disappeared on deuteration), 2.99 (1H d, J 4.8 Hz, OH; disappeared on deuteration); $J_{5_1,5_2}$ 9.0 Hz, $J_{4,5_1}$ 2.5 Hz, $J_{4,5_2}$ 4.4 Hz, $J_{2_1',2_2'}$ 9.8 Hz. Irradiation at τ 5.32 (H-3, 4 and $2_2'$), two quartets at τ 5.90 (H-5_1) and 5.55 (H-5_2) collapsed to an AB quartet (J 9.0 Hz) and a doublet at τ 5.65 (H-$2_1'$) collapsed to a singlet. Irradiation at τ 5.90, a quartet at τ 5.55 (H-5_2) collasped to a doublet (J ~5 Hz).

NMR spectrum for **III**$_b$ at 100 MHz in pyridine-d_5. τ: 8.54 (3H s, C-CH_3), 7.47 and 7.29 (each 1H doublet, forming an AB quartet centered at τ 7.38, $J_{A,B}$ 13.8 Hz, H-$4_1'$, $4_2'$), 6.65 (3H s, OCH_3); 5.91 (1H q, H-5_1), 5.58 (1H q, H-5_2); 5.74 (1H d, H-$2_1'$), 5.12 (1H d, H-$2_2'$); 5.46 (1H q, H-3), 5.28 (1H m, H-4), 3.12 (1H d, J ~3 Hz, C(4)-OH), 2.77 (1H, J ~5 Hz, C(3)-OH, overlapped with one of the signals caused by pyridine); $J_{2_1',2_2'}$ 9.0 Hz, $J_{4,5_1}$ 3.5 Hz, $J_{4,5_2}$ 5.3 Hz, $J_{5_1,5_2}$ 9.0 Hz. Irradiation at τ 5.28 (H-4) two quartets at τ 5.91 (H-5_1) and 5.58 (H-5_2) collapsed to an AB quartet. Irradiation at τ 5.12 (H-$2_2'$) a doublet at τ 5.74 (H-$2_1'$) collapsed to a singlet. Irradiation at τ 3.12, multiplet at τ 5.28 (H-4) collapsed to a quintet in appearance. Irradiation at τ 2.77, unresolved quartet at τ 5.46 (H-3) collapsed to a sharp doublet ($J_{3,4}$ 2.5 Hz).

Diacetates (**IV**-Mixture) of **III**$_a$ and **III**$_b$: A mixture of **III**$_a$ and **III**$_b$ (72 mg) was acetylated in an usual manner with acetic anhydride and pyridine. The reaction mixture, after coevaporation with toluene, was dissolved in chloroform, and the solution was washed with sodium bicarbonate solution and with water, dried over sodium sulfate and evaporated to give an EHRLICH-active syrup (93 mg, 92 %). Rf (with benzene-ether): 0.43 (major) and 0.36 (minor), $[\alpha]_D^{29}$ −20° (c 1, methanol).

Anal. Found: C 53.78, H 6.98, O 38.54.
Calcd. for $C_{13}H_{20}O_7$: C 54.16, H 6.99, O 38.85 %.

IR spectrum: 2950(w), 1750, 1432(w), 1373, 1225~1240, 1186, 1040, 841(w).

NMR spectrum at 100 MHz in pyridine-d_5. τ: 8.51 and 8.56 (3H singlets in the ratio of 2.5:1, C-CH_3), 8.02 and 8.01 (3H singlets in the ratio of 2.5:1, OAc), 7.98 (3H s, OAc), 7.80 and 7.43 (each approximately 2.5/3.5 H doublet, forming an AB quartet

centered at τ 7.62, J 13.8 Hz, H-$4_1'$, $4_2'$ in major component), 7.67 and 7.50 (each approximately 1/3.5 H doublet, forming an AB quartet centered at τ 7.58, J 14 Hz, C(3')H_2 in minor component), 6.80 and 6.72 (3H singlets, 2.5 : 1 OCH_3), 6.17 and 6.13 (each quartet in the strength ratio of approximately 2.5 : 1 (total 1H), H-5_1), 5.85 (2H triplet in appearance, the inner peak being strong, J 10Hz, H-$2_1'$, $2_2'$), 5.65 (major) 5.71 (?) (minor) (each quartet (total 1H), H-5_2), 4.68 (1H m, H-4), 4.31 and 4.47 (each doublet in the strength ratio of approximately 2.5 : 1 (total 1H), H-3); $J_{3,4}$ (major) ~1.5 Hz, $J_{3,4}$ (minor) 2 Hz, $J_{4.5_1}$ 3.2 Hz, $J_{4,5_2}$ 5.5 Hz, $J_{5_1,5_2}$ 10.5 Hz.

2'-Epimeric Mixture of 3,4-Dihydroxy-2-hydroxymethyl-2-(2'-hydroxypropyl)tetrahydrofuran (V-Mixture): To an aqueous solution (2 ml) of sphydrofuran (150 mg) sodium borohydride (19 mg) was added and the solution was allowed to stand at room temperature for 1 hour. On TLC with chloroform-methanol (7 : 1), sphydrofuran (Rf 0.23) had disappeared and products (Rf 0.18) appeared. After addition of acetone, the solution was passed through a column of Dowex 50W×2 (H form) with the aid of water. The eluate was evaporated and the residue was coevaporated with methanol several times to remove boric acid. The residue was chromatographed on a column of Dowex 1×2 (OH form) with water. The fraction containing **V** was evaporated to give a syrup; 130 mg (86 %), $[\alpha]_D^{20}$ −6° (c 1, methanol). The attempted separation of the mixture to each isomer was unsuccessful.

Anal. Found: C 49.70, H 8.50, O 41.38.
Calcd. for $C_8H_{16}O_5$: C 49.99, H 8.39, O 41.62 %.

NMR spectrum at 100 MHz in dimethylsulfoxide-d_6. τ: 8.93 and 8.94 (3H doublets in the integration ratio of approximately 2.5 : 1, J 6 Hz, CH-C$\underline{H}_3$), 8.6~8.1 (2H m, C-C$\underline{H}_2$-CH-).

Tetraacetates (VI-Mixture) of V-Mixture: **V**-Mixture (30 mg) was acetylated with acetic anhydride in pyridine to give a colorless syrup (**VI**-Mixture), 42 mg (75 %), Rf 0.5 (benzene - ether, 1 : 1), $[\alpha]_D^{20}$ −8.6° (c 1, methanol).

Anal. Found: C 53.27, H 6.75.
Calcd. for $C_{16}H_{24}O_9$: C 53.33, H 6.71 %.

NMR spectrum at 100 MHz in pyridine-d_5. τ: 8.73 and 8.75 (3H doublets in the integration ratio of approximately 2.5 : 1, J 6 Hz, CHC$\underline{H}_3$), 8.00~7.94 (12H, five peaks, OAc), 8.2~7.5 (2H m, CH_3CHC$\underline{H}_2$-C); 6.15~5.3: 4H, nineteen peaks which were analyzed as follows: τ 6.06 (1H q, J 10 and 3.5 Hz, H-5_1), τ 5.64 and 5.58 (1H quartets, the former being more intense; each had J 10 and 6 Hz; H-5_2); τ 5.70 and 5.80 (1H doublets, the former being more intense; each had J 11.5 Hz, AcOC$\underline{H}_2$-C); τ 5.47 and 5.41 (1H doublets, the former being more intense; each had J 11.5 Hz, AcOC$\underline{H}_2$-C); τ 4.75~4.45 (2H m, H-4 and AcO-C$\underline{H}$(CH_3)-C), τ 4.29 and 4.17 (1H doublets, J ~3 Hz, H-3).

2'-Epimers of 3,4-Dihydroxy-2-(2'-hydroxypropyl)-2-trityloxymethyltetrahydrofurans (**VII_a** and **VII_b**): To a solution of **V**-Mixture (101 mg) in pyridine (4 ml), trityl chloride (260 mg) was added and the solution was heated at 45°C for 100 hours. After addition of a small amount of water and subsequent concentration to a small volume, the solution was poured into chloroform and the organic layer was washed successively with water, potassium bisulfate solution and water, dried over sodium sulfate and evaporated. The resulting syrup was loaded on a small column of silica gel (Mallinckrodt AR-100), washed with benzene and eluted with benzene-acetone (4 : 1). The syrup obtained (160 mg, 70 %) was then chromatographed on a column of silica gel (Mallinckrodt CC-7, 1.5×25 cm) with chloroform-methanol (15 : 1). A fraction (27~31 ml) was evaporated to give a syrup (**VII_b**), 23.4 mg, Rf 0.35 (chloroform-methanol 15 : 1), $[\alpha]_D^{25}$ −14° (c 1, methanol). Fraction (31~35 ml) gave a mixture of **VII_a** and **VII_b** (70.2 mg). Another fraction (35~61 ml) was evaporated to give a syrup (**VII_a**), which crystallized on standing, 52 mg, Rf 0.31, $[\alpha]_D^{25}$ −4° (c 1, methanol).

Anal. Found: VI_a: C 74.55, H 6.86; VI_b: C 74.33, H 7.18.
Calcd. for $C_{27}H_{30}O_5$: C 74.63, H 6.96 %.

NMR spectra at 60 MHz: VI_a (in $CDCl_3$): τ: 8.90 (3H d, J 6 Hz, CHC$\underline{H}_3$), 2.9~2.5 (15H m, trityl). VI_b (in $CDCl_3$): τ: 8.92 (3H d, J 6 Hz, CHC$\underline{H}_3$), 2.9~2.6 (15H m, trityl).

Dimethyl Ketals ($VIII_a$ and $VIII_b$) from V-Mixture: To a solution of V-Mixture (702 mg) in dry acetone (15 ml), anhydrous cupric sulfate (1.75 g) was added and the mixture was stirred at room temperature. On TLC with benzene - ethanol (8 : 1), after 30 minutes, a pair of products ($VIII_a$ (major), Rf 0.29, and $VIII_b$ (minor), Rf 3.33) appeared accompanied with an intermediate (Rf 0.18). On TLC as visualized with sulfuric acid, the ratio of $VIII_a$+$VIII_b$, the intermediate and the starting materials (Rf 0.04) was approximately 1 : 1 : 1. When the reaction was carried out for 2 hours, the spots of $VIII_a$ and $VIII_b$ became dominant on TLC. The reaction was stopped after 24 hours and the mixture was filtered. After evaporation, half of the resulting syrup (830 mg) was applied on a column of silica gel (Mallinckrodt CC7, 2.3×57.5 cm) and developed with benzene - ethanol (8 : 1) containing 0.1 % triethylamine. A fraction (273~331 ml) was evaporated to give crystals ($VIII_b$), 87 mg (20 %), which was recrystallized from acetone-benzene-n-hexane; m.p. 121~122°C, $[\alpha]_D^{27}$ −25° (c 1, pyridine). From a fraction (331~372 ml) a mixture of $VIII_a$ and $VIII_b$ (120 mg) was obtained. Another fraction (372~467 ml) was evaporated to give a syrup ($VIII_a$), 124 mg (29 %), which crystallized on standing. Recrystallization from acetone - benzene - n-hexane gave m.p. 74.5~75.5°C, $[\alpha]_D^{27}$ +32° (c 1, pyridine).

Anal. Found: $VIII_a$: C 56.81, H 8.81; $VIII_b$: C 57.09, H 8.75.
Calcd. for $C_{11}H_{20}O_5$: C 56.88, H 8.68 %.

NMR spectrum of $VIII_a$ at 100 MHz in pyridine-d_5. τ: 8.60 (3H d, J 6.2 Hz, CHC$\underline{H}_3$), 8.54 and 8.52 (each 3H s, isopropylidene), 7.76 (H-1_1′′) and 7.62 (H-1_2′′) (each 1H quartet, forming the AB part of an ABX system), 5.86 (H-6_1′) and 5.70 (H-6_2′) (each 1H doublet, forming an AB quartet), 5.82 (H-5_1) and 5.59 (H-5_2) (each 1H quartet, forming the AB part of an ABX system), 5.48 (1H unresolved multiplet, which became a better resolved multiplet when D_2O was added, H-2′′), 5.44 (1H s, H-3), 5.33 (1H broad signal, which became a quartet) (J ~1.5 and ~4 Hz) when D_2O was added, H-4), 2.97 (OH), 4.2 (OH); $J_{3,4}$~0 Hz, $J_{4.51}$ 1.5 Hz, $J_{4.52}$ 4.2 Hz, $J_{51.52}$ 9.5 Hz, $J_{61',62'}$ 11.8 Hz, $J_{11'',2''}$ 8.8 Hz, $J_{12'',2''}$ 3.2 Hz, $J_{11'',12''}$ 14.3 Hz.

NMR spectrum of $VIII_b$ at 100 MHz in pyridine-d_5. τ: 8.67 (3H d, J 6.1 Hz, CHC$\underline{H}_3$), 8.55 and 8.54 (each 3H s, isopropylidene), 7.93 (H-1_1′′) and 7.57 (H-1_2′′) (each 1H quartet, forming the AB part of an ABX system), 6.04 (2H singlet in appearance, H-6_1′, 6_2′), 5.77 (H-5_1) and 5.67 (H-5_2) (each 1H quartet, forming the AB part of an ABX system), 5.5 (1H m, which became more resolved multiplet when D_2O was added, H-2′′), 5.37 (1H narrow multiplet, H-4), 5.34 (1H s, H-3), 2.84 (1H d, J 3.9 Hz, OH), 4.47 (1H, OH). $J_{3,4}$~0 Hz, $J_{4.51}$ ~1 Hz, $J_{4.52}$ 3.7 Hz, $J_{51.52}$ 9.5 Hz, $J_{61',62'}$ ~12 Hz, $J_{11'',2''}$ 2.5 Hz, $J_{12'',2''}$ 9.7 Hz, $J_{11'',12''}$ 14.7 Hz.

Diacetates (IX_a and IX_b) of Dimethyl Ketals ($VIII_a$ and $VIII_b$): Compound $VIII_a$ (37.1 mg) was acetylated with acetic anhydride and pyridine. Diacetate (IX_a) was obtained as a syrup 41 mg (81 %), Rf 0.55 (benzene - ether, 2 : 1), $[\alpha]_D^{27}$ +19° (c 1, benzene). Compound $VIII_b$ (40.5 mg) was treated likewise to give the corresponding diacetate as a syrup, 47 mg (85 %), Rf 0.56 (benzene - ether, 2 : 1), $[\alpha]_D^{27}$ −41° (c 1, benzene).

Anal. Found: IX_a: C 56.61, H 7.53, O 35.25; IX_b: C 56.82, H 7.59, O 35.28.
Calcd. for $C_{15}H_{24}O_7$: C 56.95, H 7.65, O 35.40 %.

NMR spectrum of IX_a in pyridine-d_5. τ: 8.71 (3H d, J 6.2 Hz, CHC$\underline{H}_3$), 8.62 (6H s, isopropylidene); 8.07 (H-1_1′′) and 7.86 (H-1_2′′) (each 1H quartet, forming the AB part of an ABX system); 8.03 and 7.96 (each 3H singlet, OAc); 6.14 (H-6_1′) and 5.99 (H-6_2′) (each 1H doublet, forming AB quartet); 6.05 (1H doublet in appearance, H-5_1), 5.70 (1H q, H-5_2); 5.74 (1H s, H-3), 4.73 (1H q, H-4), 4.63 (1H m, H-2′′); $J_{3,4}$ ~0 Hz, $J_{4.51}$ ~1.5 Hz, $J_{4.52}$ 4.3 Hz, $J_{51.52}$ 10.5 Hz, $J_{61',62'}$ ~12 Hz, $J_{11'',2''}$ 4.5 Hz, $J_{12'',2''}$ 7.5 Hz, $J_{11'',12''}$ 14.6 Hz.

Irradiation at τ 4.73 (H-4) caused H-5_1, 5_2 to become an AB quartet. Irradiation at τ 4.63 (H-2″) caused H-1_1″, 1_2″ to become an AB quartet and CHC$\underline{H}_3$ to a singlet.

NMR spectrum of **IX**$_b$ in pyridine-d_5. τ: 8.71 (3H d, J 6.1 Hz, CHC$\underline{H}_3$), 8.65 and 8.62 (each 3H singlet, isopropyridene), 8.03 and 7.95 (each 3H singlet, OAc); 8.15 (H-1_1″) and 7.67 (H-1_2″) (each 1H quartet, forming the AB part of an ABX system); 6.30 (H-6_1′) and 6.19 (H-6_2′) (each 1H doublet, forming an AB quartet); 6.00 (H-5_1) and 5.78 (H-5_2) (each 1H quartet, forming the AB part of an ABX system); 5.56 (1H s, H-3), 4.75 (1H q, H-4), 4.66 (1H m, H-2″). $J_{3,4}$ ~0 Hz, $J_{4,5_1}$ ~1.5 Hz, $J_{4,5_2}$ 4 Hz, $J_{5_1,5_2}$ 10.5 Hz, $J_{6_1',6_2'}$ 11.5 Hz, $J_{1_1'',2''}$ 4.2 Hz, $J_{1_2'',2''}$ 7.5 Hz, $J_{1_1'',1_2''}$ 15.2 Hz. Irradiation at τ 4.75 (H-4) caused H-5_1, 5_2 to become an AB quartet. The signals assignable to H-1_1″, 1_2″ also changed. Irradiation at τ 4.66 (H-2″) caused H-1_1″, 1_2″ to become an AB quartet and CHC$\underline{H}_3$ to a singlet. Irradiation at τ 5.56 (H-3) caused H-5_1 to become a sharp quartet.

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