et que l'acide penicillique provoque des lésions histopathologiques diffuses du foie, du rein et de la thyroide, *etc.*[10]

On aussi sait que c'est une substance cytotoxique ā la concentration de 3.2 μg/mg quande on ajoute l'acide penicillique dans une culture de cellule HeLa.

A cette occasion, on a pu observer des anomalies du nuclei de ces cellules.

Nous n'avons pas encore observé de résultats qui nous permettent de relier l'acide penicillique à la cirrhose du foie quande on l'administre isolement aux animaux, mais nous pensons que l'acid penicillique joue un rôle principal dans l'intoxication par P. olivino-viride, parce que la quantité d'acide penicillique produit par ce microbe est énorme.

Des essais biologiques menés par notre équipe indiquent qu'il y a une autre substance cytotoxique, un peu plus puissante que l'acide penicillique et, nous nous occupons maintenant de l'isolement de cette toxine ā partir de métabolites du P. olivino-viride.

10) Communication privée.

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UDC 547.597.04 : 542.98

Microbial Transformation of Sesquiterpenoids. III.[1] 6β- and 7α-Hydroxylation of Guaioxide

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As reported already [3]microbial oxidation of guaioxide (I) with *Mucor parasiticus* yielded 4α-hydroxy-, 8α-hydroxy-, and 4α,8α-dihydroxy-guaioxide, all of which were useful for the structure determination of guaioxide itself. On further examination of this reaction we have isolated 6β-hydroxy- and 7α-hydroxy-guaioxide (II and III).

Compound (II), $C_{15}H_{26}O_2$, mp 160—161°, showed an infrared (IR) hydroxy-band at 3630 cm^{-1}, and on oxidation with Jones reagent gave a ketone (IV), ν_{max} 1747 (five-membered ring ketone) cm^{-1}; thus the hydroxyl group in II is secondary and located at C-2, C-3, or C-6. When passed through a column of alumina the ketone (IV) remained unchanged. Since 2-oxo-[4] and 3-oxo-guaioxide[3] are known to rearrange on alumina, the structure 6-oxoguaioxide (IV) was assigned to this ketone. Lithium aluminum hydride reduction of IV furnished predominantly an epimeric alcohol (V), mp 161—165°. The configuration of the C-6 hydroxyl groups in II and V were assigned by inspection of the nuclear magnetic resonance (NMR) spectrum and examination of molecular models of these compounds. The signal for the C-6 proton in II appeared as a singlet at τ 5.94, showing that the dihedral angle between C(6)-H and C(7)-H is about 90°. This indicates that the C(6)-H in II is α-oriented, *i.e.* the hydroxyl group at C-6 has β-configuration.

The signal pattern of the C(6)-H in V is a broad doublet (J=6 cps; τ 5.53) so that the dihedral angle between C(6)-H and C(7)-H is about 30°, indicating that the orientation of the C(6)-H in V is β. Therefore compound (V) is 6α-hydroxyguaioxide.

1) Part II: E. Funke, T. Tozyo, H. Ishii, and K. Takeda, *J. Chem. Soc.* (*C*), **1970**, 25 84.
2) Location: *Fukushima-ku, Osaka.*
3) H. Ishii, T. Tozyo, M. Nakamura, and H. Minato, *Tetrahedron*, **26**, 2751 (1970).
4) C. Ehret and G. Ourisson, *Bull. Soc. Chim. France*, **1968**, 2629.

Compound (III), $C_{15}H_{26}O_2$, mp 135°, ν_{max} 3620 (OH) cm^{-1}, resisted acetylation under mild conditions and Jones oxidation. Further, its NMR spectrum showed no>C<u>H</u>-OH signal. Thus the hydroxyl group in III is tertiary. That this is located at the 7α-position was clarified by the following experimental results (1), (2) and (3) similar to those obtained in structure determination of 7α-hydroxyliguloxide[1]: (1) The NMR signal for the methyl groups on C-4 and C-10 appeared as a pair of doublets, excluding the possibility that the hydroxyl group is attached to these two carbon atoms. (2) An attempted dehydration with thionyl chloride in pyridine was unsuccessful, indicating that the hydroxyl group must be at bridgehead 7α-position. (3) The IR spectrum of a dilute solution of III in carbon tetrachloride showed no evidence for an intramolecular hydrogen bond between the hydroxyl group in question and 5β-ethereal oxygen atom, suggesting that the hydroxyl group is not at 1β-position.

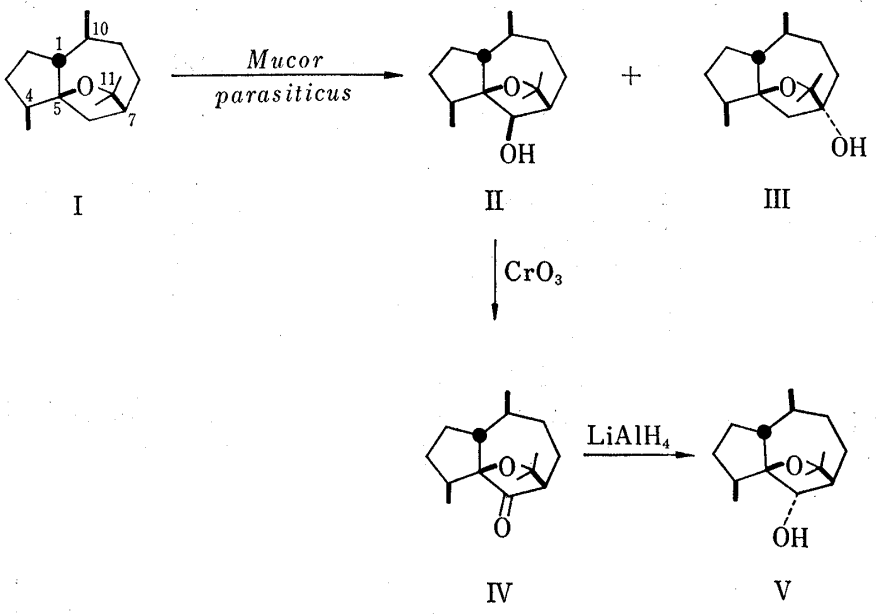

Chart 1

Experimental

Rotations were taken in dioxane. NMR spectra were recorded on a Varian A-60 spectrometer in $CDCl_3$ with TMS as internal standard.

Isolation of 6β-Hydroxyguaioxide (II) and 7α-Hydroxyguaioxide (III)——A fermentation product (14.9 g) of guaioxide (I) with *Mucor parasiticus* was chromatographed on Al_2O_3 (activity V, 500 g).[3] The fractions eluted with petr. ether–ether (98:2) and (95:5) were combined (5.1 g), and distilled at up to 140° (bath)/4 mm to give a viscous oil (790 mg). The oil was crystallized from petr. ether giving II (575 mg) as colorless plates, mp 160—161°, $[\alpha]_D^{23}$ −42.2° (c=0.985). *Anal.* Calcd. for $C_{15}H_{26}O_2$: C, 75.58; H, 11.00. Found: C, 75.56; H, 10.95. IR cm^{-1}: ν_{max} 3630 ($CHCl_3$). NMR τ: 9.10 (3H, diffuse d, C_{10}–C<u>H</u>$_3$), 8.97 (3H, d, J=7 cps, C_4–C<u>H</u>$_3$), 8.70 and 8.67 (each 3H, s, C_{11}–(C<u>H</u>$_3$)$_2$), 5.94 (1H, s, >C<u>H</u>–OH).

The middle eluate with petr. ether–ether (9:1)[3] afforded a crystalline substance (330 mg), a part of which was sublimed to give a pure sample of III as colorless plates, mp 135°, $[\alpha]_D^{21}$ +0.3° (c=0.870). *Anal.* Calcd. for $C_{15}H_{26}O_2$: C, 75.58; H, 11.00. Found: C, 75.66; H, 10.93'. IR cm^{-1}: ν_{max} 3620 ($CHCl_3$). NMR τ: 9.80 (3H, diffuse d, C_{10}–C<u>H</u>$_3$), 9.02 (3H, d, J=7 cps, C_4–C<u>H</u>$_3$), 8.89 and 8.77 (each 3H, s, C_{11}–(C<u>H</u>$_3$)$_2$).

6-Oxoguaioxide (IV)——Jones reagent (0.1 ml) was added to a cooled solution of II (50 mg) in acetone (1 ml) and the mixture was stirred for 5 min at room temperature. The reaction mixture was worked up in the usual manner to give a pale yellow oil (50 mg), which was distilled at 80° (bath)/4 mm yielding IV (45 mg) as a colorless viscous oil, $[\alpha]_D^{23}$ −60.8° (c=0.936). *Anal.* Calcd. for $C_{15}H_{24}O_2$: C, 76.22; H, 10.24. Found: C, 76.69; H, 10.22. IR cm^{-1}: $\nu_{C=O}$ 1747 ($CHCl_3$). NMR τ: 9.10 (3H, diffuse d, C_{10}–C<u>H</u>$_3$), 9.07 (3H, d, J=6 cps, C_4–C<u>H</u>$_3$), 8.90 and 8.62 (each 3H, s, C_{11}–$(CH_3)_2$).

6α-Hydroxyguaioxide (V)——A solution of IV (80 mg) in dry ether (3 ml) was added portionwise to a suspension of $LiAlH_4$ (45 mg) in dry ether (5 ml), and the mixture was stirred for 1 hr at room temperature.

The reaction mixture was treated in the usual manner and the crystalline product (75 mg) thus obtained was sublimed to give a pure sample of V as colorless prisms, mp 161—165°, $[\alpha]_D^{23}$ −5.8° (c=0.791). *Anal.* Calcd. for $C_{15}H_{26}O_2$: C, 75.58; H, 11.00. Found: C, 75.76; H, 11.01. IR cm^{-1}: ν_{max} 3635 ($CHCl_3$). NMR τ: 9.12 (3H, d, J=5 cps, C_{10}-C<u>H</u>$_3$), 9.02 (3H, d, J=7 cps, C_4-C<u>H</u>$_3$), 8.82 and 8.63 (each 3H, s, C_{11}-(C<u>H</u>$_3$)$_2$), 5.53 (1H, broad d, J=6 cps, >C<u>H</u>-OH).

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UDC 547.92.04 : 547.918.07

Studies on Steroid Conjugates. V. Synthesis of 16-Epiestriol 3-Glucuronide[1]

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As a part of our program dealing with the studies on steroid conjugates we reported previously the synthesis of 16-epiestriol (estra-1,3,5(10)-triene-3,16β,17β-triol) 16- and 17-glucuronides.[3] Three possible monoglucuronides have become requisite for us to explore the estrogen conjugates in pregnancy urine and examine the multiplicity of the transferase which catalyzes the formation of the glucuronoside linkage. In this paper we wish to report the preparation of remaining 16-epiestriol 3-monoglucuronide.

First, 16-epiestriol (IIIa) was led to the acetonide to protect the 16,17-*cis*-glycol structure. However, the attempt to introduce the glucuronyl moiety employing Koenigs–Knorr reaction[4] resulted in failure.

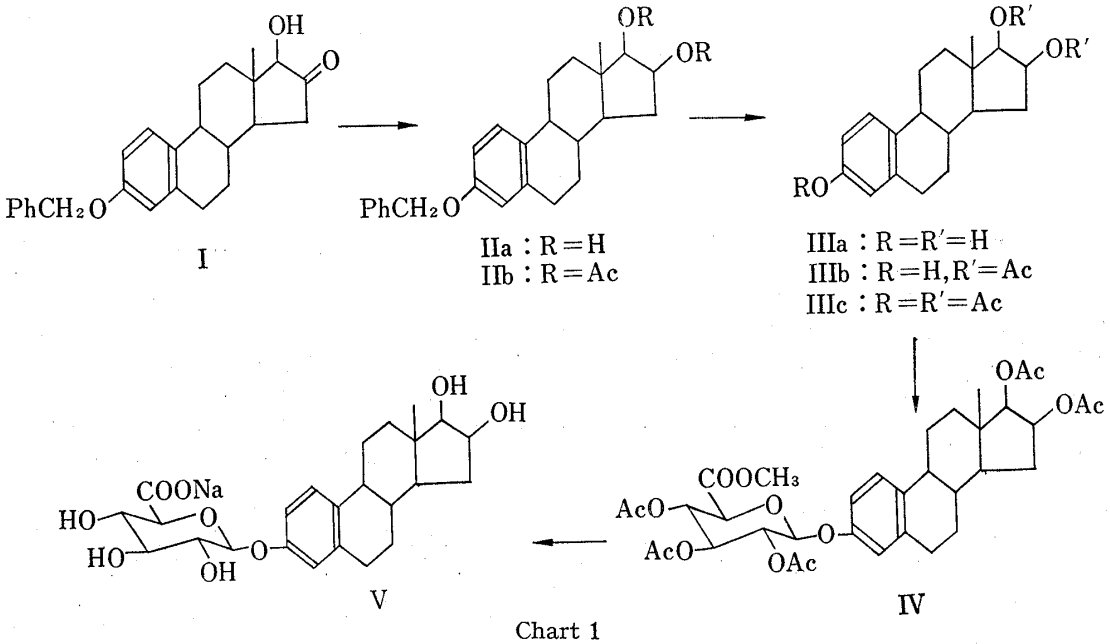

Chart 1

1) This paper constitutes Part XLIV of the series entitled, "Analytical Chemical Studies on Steroids"; Part XLIII: T. Nambara, H. Hosoda, M. Usui, and T. Anjyo, *Chem. Pharm. Bull.* (Tokyo), **19**, 612 (1971).
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4) H.H. Wotiz, E. Smakula, N.N. Lichtin, and J.H. Leftin, *J. Am. Chem. Soc.*, **81**, 1704 (1959).