treated with EA_3 and the antagonist together, the inhibitory effect was remarkably high, when 3-NH_2-POL was used as an antagonist, complete tumor regression increased as shown in Table I.

Table II showed the histamine and serotonin values of tumor tissues at the end of 4 weeks after transplantation. The histamine and serotonin values decreased under vitamin B_6 deficient diet without EA_3 administration, and this tendency was an expected result. When treated with EA_3 in dosage of 5 mg/kg/day, the histamine value increased remarkably and the same tendency was found when treated with EA_3 and the antagonists together. However, this tendency was not observed in dosage of 1 mg/kg/day of EA_3. No clear correlation between antitumor effect and histamine value in the tumor tissues was found at 4 weeks after transplantation.

The constant tendency was not obtained in the histamine value at different dosage of EA_3 administration, but the serotonin value highly increased at both dosages of 5 mg/kg/day and 1 mg/kg/day of EA_3 in comparison with that of the control.

Thus it may be considerable that there is some correlation between the host-mediated antitumor effect and the serotonin value in tumor tissue. It may also be of interest that serotonin itself has some inhibitory effect on the growth of the solid tumor of sarcoma 180.[7]

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7) B. Sokoloff, "Recent Results in Cancer Research," ed. by P. Rentchnick, Vol. 15, Springer- Verlag, Berlin, 1968, p. 20.

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11-O-Galactosyl-nogiragenin, a Prosapogenol of *Metanarthecium luteo-viride* Maxim. obtained by Soil Bacterial Hydrolysis

In search of the genuine sapogenols of *Metanarthecium luteo-viride* Maxim. (Japanese name: nogiran), whose steroidal sapogenols were extensively investigated by Takeda, *et al.*,[1] we have been examining the usefulness of soil bacterial hydrolysis method[2] for the glycosides

1) *a*) K. Hamamoto, *Chem. Pharm. Bull.* (Tokyo), **9**, 32 (1961); *b*) K. Takeda, T. Okanishi, H. Osaka, A. Shimaoka, and N. Maezono, *ibid.*, **9**, 388 (1961); *c*) H. Minato and A. Shimaoka, *ibid.*, **11**, 876 (1963); *d*) K. Igarashi, *ibid.*, **9**, 722 (1961); *e*) K. Takeda, T. Okanishi, K. Igarashi, and A. Shimaoka, *Tetrahedron*, **15**, 183 (1961).

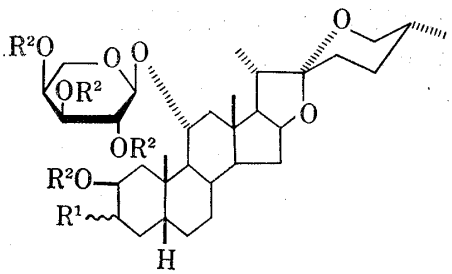
2) *a*) I. Yosioka, M. Fujio, M. Osamura, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 6303; *b*) I. Yosioka, T. Sugawara, K. Yoshikawa, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 2450 (1972), and the preceding papers of the series cited therein.

of the plant. As reported previously,[3] two prosapogenols: 11-O-[tri-O-acetyl-α-L-arabinosyl]-2-O-acetyl-3-epimetagenin (Ia) and 11-O-[tri-O-acetyl-α-L-arabinosyl]-2-O-acetyl-metagenin (Ib) were isolated from the aerial part by virtue of the microbiological method and they are characteristic due to the possession of a glycoside linkage at C-11 of the steroidal skeleton.

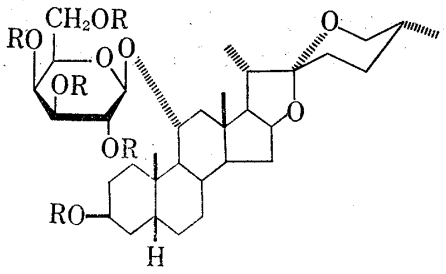
In the present communication, we wish to report the isolation and structure elucidation of 11-O-galactosyl-nogiragenin (IIa), another example of a prosapogenol having a glycoside linkage at C-11, through the application of soil bacterial hydrolysis method to the glycosides portion of the subterranean part.

A total hydrolysis product obtained by cultivation using a soil bacterial strain (YSB-27) as described previously[2a] was divided into the ether soluble and *n*-butanol soluble portions (55.4% and 28.8% from the total hydrolysis product). The ether soluble portion was treated with acetic anhydride and pyridine and chromatographed on silica gel to afford an acetylated prosapogenol (IIb) in 5.9% yield (from the total acetate mixture).

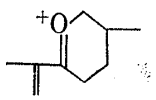
IIb, $C_{43}H_{64}O_{14}$,[4] mp 229—232°, $[\alpha]_D^{18}$ −63° ($CHCl_3$), shows the infrared (IR) absorption bands at 1762, 1741 (sh), 1738, 1250, 1239, 1220 cm^{-1} (acetate) and 980, 919<898, 861 cm^{-1} (25R-spiroketal),[5] but no hydroxyl band. It shows five acetoxyl signals at δ 1.95 (3H, s), 1.98 (3H, s), 2.00 (6H, s), 2.12 (3H, s) in its proton magnetic resonance spectrum (90 MHz, $CDCl_3$). Mass spectrum of IIb reveals the existence of a spiroketal moiety by a base peak at *m/e* 139 (IIIa)[6a] and that of tetra-O-acetyl-hexaaldopyranosyl moiety by a fragment ion peak at *m/e* 331 (IIIb, 73%).[6] On alkaline treatment, IIb gave a desacetyl derivative (IIa),



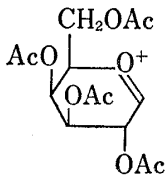
Ia : R^1=3α-OH, R^2=Ac
Ib : R^1=3β-OH, R^2=Ac
Ic : R^1=3α-OH, R^2=H
Id : R^1=3β-OH, R^2=H



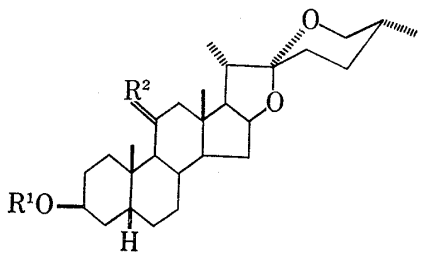
IIa : R=H
IIb : R=Ac
IIc : R=Me



IIIa : *m/e* 139



IIIb : *m/e* 331



IVa : R^1=H, R^2=α-OH,β-H
IVb : R^1=Me, R^2=α-OH,β-H
IVc : R^1=Me, R^2=O

Chart 1

3) I. Yosioka, K. Imai, and I. Kitagawa, *Tetrahedron Letters*, **1971**, 1177.
4) The new compounds given with the molecular formulae gave the satisfactory analytical values.
5) M.E. Wall, C.R. Eddy, M.L. McClennan, and M.E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952).
6) *a*) H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day Inc., San Francisco, 1964, p. 113; *b*) *Idem, ibid.*, p. 207.

$C_{33}H_{54}O_9$ (amorphous), $[\alpha]_D^{10}$ −71.6° (pyridine), which on further acid hydrolysis yielded nogiragenin (IVa)[1b] and D-galactose. Penta-O-methyl derivative (IIc) of IIa prepared by the Hakomori's procedure[7] shows no hydroxyl absorption band in its IR spectrum. It shows a doublet at δ 4.26 (J=7.5 Hz) assignable to an anomeric proton thus corroborating a β-galactoside linkage in IIa.

Acid treatment of IIc afforded nogiragenin monomethyl ether (IVb), $C_{28}H_{46}O_4 \cdot 1/2H_2O$, mp 166.5—168.5°, $[\alpha]_D^{18}$ −41.5° ($CHCl_3$), which was then subjected to chromium trioxide-pyridine oxidation to furnish a monoketone (IVc), $C_{28}H_{44}O_4$, mp 153—155.5°, $[\alpha]_D^{18}$ −21.0° ($CHCl_3$); ORD (c=0.139, dioxane) $[\Phi]^{25}$ (nm): 0° (344), +852° (329) (peak), +386° (320) (sh), 0° (314), −2475° (286) (sh), −2571° (284) (trough); CD (c=0.0031, dioxane) $[\theta]^{25}$ (nm): 0 (344), +1230 (323) (sh), +1909 (312) (maximum), +1866 (308), +1909 (304) (maximum), 0 (256). The positive Cotton effect demonstrates the location of a carbonyl function of IVc being at C-11[8] and therefore the structure of nogiragenin monomethyl ether is expressed as IVb. Consequently, the prosapogenol peracetate is formulated as IIb. Thin-layer chromatographic examination of the original ether soluble portion in comparison with IIa and IIb discloses that the ether extract contains IIa and its partially acetylated derivatives but does not contain IIb. The rest of the components mainly comprises the partially acetylated derivatives of 11-O-α-L-arabinosyl-3-epimetagenin (Ic)[3] and 11-O-α-L-arabinosyl-metagenin (Id).[9]

Together with the previous elucidation of 11-O-arabinosyl derivatives (Ia—Id),[3,9] the present observation offers an additional evidence concerning the complete structures of glycosides in *M. luteo-viride*, which are currently under investigation.

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7) S. Hakomori, *J. Biochem.* (Japan), **55**, 205 (1964).
8) K. Takeda and H. Minato, *Steroid*, **1**, 345 (1963).
9) Presented at the 22nd Annual Meeting of Kinki Branch, Pharmaceutical Society of Japan, Osaka, November 1972, Abstract Papers, p. 38. The details will be published in a future paper.