was distilled off. The residue was distilled under reduced pressure to give yellow orange oil, bp 78—81°/2 mmHg. IR $\nu_{max}^{liq.}$ cm^{-1}: 1745, 1145 ($COOC_2H_5$), 1395, 903 (NNO). NMR τ ($CDCl_3$): 8.53 (t, CH_3), 5.53 (q, CH_2), 6.60 (t, $ClCH_2$), 5.98 (t, CH_2N). Yield, 16.25 g (90%). *Anal.* Calcd. for $C_5H_9O_3N_2Cl$: C, 33.25; H, 5.02; N, 15.51. Found: C, 32.89; H, 4.88; N, 15.47.

N-(2-Bromoethyl)-N-nitrosourethan (IXb): Orange red oil, bp 86°/2 mmHg. IR $\nu_{max}^{liq.}$ cm^{-1}: 1750, 1140 ($COOC_2H_5$), 1380, 855 (NNO). NMR ($CDCl_3$): 8.50 (t, CH_3), 5.45 (q, CH_2), 6.72 (t, $BrCH_2$), 5.88 (t, CH_2N). Yield, 91%. *Anal.* Calcd. for $C_5H_9O_3N_2Br$: C, 26.68; H, 4.03; N, 12.45. Found: C, 26.78; H, 4.02; N, 12.30.

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Soil Bacterial Hydrolysis leading to Genuine Aglycone. VI.[1] On Stevioside

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As reported previously,[1,3] the soil bacterial hydrolysis method developed in this laboratory has been shown to be a useful procedure in the structure elucidation of the genuine triterpenoid sapogenols. As an extention of these studies, we have applied the microbiological method to the diterpenoid glycoside stevioside (I). The usefulness of the method is demonstrated in this paper.[4]

The structure of the aglycone steviol (II), obtained from stevioside (I), was established by Mossetig, *et al.*,[5] and the sugar portion of the glycoside was elucidated by Fletcher and his co-workers.[6] On acid treatment, both stevioside and steviol afforded isosteviol (III), possessing a rearranged skeleton, while by snail enzyme hydrolysis, stevioside and steviolbioside (IV) were shown to give steviol as the sole hydrolysate. Also, it was noted that stevioside was unattacked by emulsin, rhamnodiastase, air-dried brewer's yeast, and powder of *Aspergillus niger*. Afterwards, however, it was found[7] that a commercial pectinase preparation is suitable for the hydrolysis of the glycoside to give steviol.

The culture broth obtained by cultivation of a soil bacterial strain (YSB-9), which was selected as described before,[8] was extracted with ether and *n*-butanol successively. From the ether extract was obtained a minor hydrolysate, mp 207—208°, whose infrared (IR) spectrum and behavior on thin-layer chromatography (TLC) resembled that of steviol. A direct comparison of the substance with steviol showed the sample to be identical to the authentic compound.

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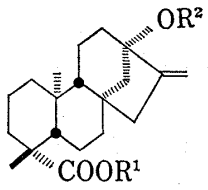
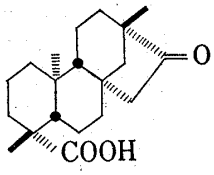


I: $R^1=\beta$-D-glucose, $R^2=\beta$-D-glucose$^{2}_{1}$-β-D-glucose stevioside
II: $R^1=R^2=H$ steviol
IV: $R^1=H$, $R^2=\beta$-D-glucose$^{2}_{1}$-β-D-glucose steviolbioside



III: isosteviol

Chart 1

From the *n*-butanol soluble portion was obtained the major product, mp 187—188°, whose IR spectrum showed the presence of a carboxylic acid function. Methylation of the product with diazomethane gave a methyl ester, whereas acetylation of the product afforded a hepta-acetate as revealed by its proton magnetic resonance (PMR) spectrum. This evidence led to the assumption that the major product is steviolbioside (IV),[6] which was verified by direct comparison of the product with the latter prepared from stevioside according to the method of Fletcher, *et al.*[6] Minute examination of the total hydrolysate disclosed that isosteviol was not formed in our soil bacterial hydrolysis. The present result extends the utility of the soil bacterial hydrolysis method to a diterpenoid glycoside. In addition, it is interesting to point out that this present hydrolysis method has left unattacked most of the glycoside linkage connected to the tertiary hydroxyl function of the aglycone, which was experienced in the previous paper.[1]

Experimental[9]

Soil Bacterial Hydrolysis of Stevioside (I)——A soil bacterial strain (YSB-9, unidentified), which was selected by repeated cultivation on a synthetic medium containing stevioside as an only carbon source as described previously,[8] was cultivated on the same synthetic medium (two flasks of 500 ml medium each containing 1.5 g of stevioside)[10] at 31° for 5 days. Since TLC of the culture broths showed complete hydrolysis of stevioside, the total culture broths were extracted with ether to give an extract of 0.11 g (i) and then with *n*-butanol saturated with water. The residue obtained by evaporation of aqueous *n*-butanol *in vacuo* was treated with MeOH to give a soluble portion of 0.73 g (ii) and a less soluble portion of 1.17 g (iii). TLC (SiO_2, $CHCl_3$: MeOH=20: 1) of the ether extract (i) showed that it contained mostly steviol (II). TLC (developing with the upper layer of *n*-BuOH: AcOH: water=4: 1: 5 mixture) of the two fractions (ii and iii) revealed that ii mainly consisted of steviolbioside (IV) and other minor components (unidentified) while iii was almost pure steviolbioside. From an additional cultivation (three flasks of 500 ml medium), two flasks were treated as above to give an ether extract of 0.09 g (i) and a total *n*-butanol extract of 1.87 g (ii and iii), and another flask afforded steviolbioside (0.75 g) only, by filtration of the culture broth.

Identification of the Hydrolysate——The residue from the ether extract (i, 0.11 g) obtained above was crystallized from MeOH to give a substance (36 mg), mp 207—208°, IR ν_{max}^{KBr} cm^{-1}: 3470—3400 (OH), 1715 (sh), 1695 (COOH), 880 (>C=CH_2), which was identified with steviol (II) by mixed mp, IR (KBr), and TLC.

The insoluble MeOH portion (iii, 150 mg) was crystallized from MeOH to give colorless needles (81 mg), IR ν_{max}^{KBr} cm^{-1}: 3450 (br) (OH), 1700 (COOH), 895 (>C=CH_2), which was identified as steviolbioside (IV) prepared below by mixed mp, IR (KBr), and TLC.

To a solution of IV (53 mg) in MeOH (15 ml) was added ethereal diazomethane and the product was crystallized from MeOH to give the methyl ester (37 mg), mp 163.5—164°. *Anal.* Calcd. for $C_{33}H_{52}O_{13}\cdot H_2O$: C, 58.74; H, 8.07. Found: C, 58.84; H, 8.29. IR ν_{max}^{KBr} cm^{-1}: 3450—3400 (OH), 1720 ($COOCH_3$), 1660, 895 (>C=CH_2).

9) The following instruments were used for the physical data. Melting points: Yanagimoto Micro-melting-point Apparatus (a hot-stage type): IR spectra: Hitachi EPI-S2 IR Spectrometer; PMR spectra: Hitachi H-60 NMR Spectrometer.

10) TLC examination of the sterilized medium disclosed a minor formation of steviolbioside during the sterilization (at 120°, 2 atm, for 20 min).

Acetylation of IV (101 mg) with Ac_2O (2 ml) and pyridine (5 ml) at room temperature overnight followed by usual work-up furnished a heptaacetate (117 mg) which was purified by ether–*n*-hexane (amorphous). *Anal.* Calcd. for $C_{46}H_{64}O_{20}$: C, 58.96; H, 6.89. Found: C, 58.77; H, 6.63. PMR ($CDCl_3$) τ: 8.95 (3H, s), 8.74 (3H, s), 8.01, 7.94, 7.90 (totally 21H, all s).

Steviolbioside (IV) from Stevioside (I)——A mixture of I (200 mg) in aq. 10% KOH (5 ml) was heated at 100° for one hour. After cooling, the reaction mixture was acidified with glacial acetic acid and a product was crystallized from MeOH to give IV (75 mg), mp 184—185°. IR ν_{max}^{KBr} cm^{-1}: 3450 (br) (OH), 1700 (COOH), 895 (>C=CH_2). In lit.[6]: mp 188—192° (corr.), IR ν_{max} μ: 3.65—4.25 (carboxyl-hydroxyl), 5.92, 6.03 (w) (carboxyl).

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Synthetic Studies on Anthracyclinones. XII.[1] Synthesis of 8-Ethyl-1,6,11-trihydroxynaphthacenequinone

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8-Ethyl-1,6,11-trihydroxynaphthacenequinone (I)[3] is the important substance known as bisanhydro-γ-rhodomycinone[4] or bisanhydrodecarbomethoxy-ε-rhodomycinone[5] and is considered to be a potential starting material for the synthesis of bisanhydrodaunomycinone (II).[6]

In the course of the synthetic studies of II, it was needed for the present authors to prepare a considerable amount of I by a rather simple procedure involving a explicit characterization of the intermediates.

Compound (I) was first synthesized by Brockmann, *et al.*[3] starting from Friedel-Crafts condensation of 3-hydroxyphthalic anhydride (III) with 7-ethyl-1-naphthol[3b] (IV) and with 6-ethyl-1-naphthol (V) without separation or characterization of the isomeric condensed products.

Recently, there has been clarified[7] the behavior of the condensation of III or 3-methoxyphthalic anhydride (VI) with α-naphthol. According to the result, the condensation of V

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