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Saponin and Sapogenol. XXIX.[1] Selective Cleavage of the Glucuronide Linkage in Oligoglycosides by Anodic Oxidation

Isao Kitagawa, Toshiyuki Kamigauchi, Hidenobu Ohmori, and Masayuki Yoshikawa

Faculty of Pharmaceutical Sciences, Osaka University[2]

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In the course of studies aimed at finding a new selective cleavage method for the glucuronide linkage in glucuronide-saponins, an electrolytic oxidation reaction has been found to be useful as an initial reaction for the desired selective cleavage method. The new cleavage method comprises an anodic oxidation of glucuronide-saponin, by which the carboxyl function is converted to an acetoxyl moiety, and a subsequent alkali treatment. It has also been found that the anodic oxidation causes selective oxidation of axial hydroxyl groups and allylic oxidation in the sapogenol moiety.

Keywords——anodic oxidation; allylic oxidation; sakuraso-saponin; soyasaponin I; desacyl-jegosaponin; glycyrrhizin; glucuronide-saponin; glucuronide linkage; soyasapogenol B; protoprimulagenin A

During the course of studies in pursuit of selective cleavage methods for specific glycoside linkages in oligoglycosides such as saponins,[3,4] we have developed three new degradation methods, *i.e.* photolysis,[3,5] lead tetraacetate degradation,[3,6] and acetic anhydride-pyridine degradation,[3,7] by which the glucuronide linkage in oligoglycosides is selectively cleaved. Among these methods, in the lead tetraacetate degradation, the cleavage of a glucuronide linkage in an oligoglycoside is accomplished by initial conversion of the carboxyl moiety to an acetoxyl moiety *via* oxidative decarboxylation, followed by alkali treatment.[6] It has been presumed therefore that any procedure which replaces the carboxyl moiety in uronic acid with a labile function may be useful as an initial reaction step for directing selective cleavage of the glucuronide linkage. After several attempts, we have found that an electrolytic decarboxylation reaction is suitable for this purpose. This paper describes in detail a new selective cleavage method for the glucuronide linkage in oligoglycosides, initiated by anodic oxidation. Furthermore, some interesting electrolytic reactions, including selective oxidation of axial hydroxyl groups and allylic oxidation, are also described.[8]

Electrolytic Decarboxylation of Methyl Glucuronide

Various studies have been undertaken in connection with electrolytic decarboxylation.[9]

1) Part XXVIII: I. Kitagawa, M. Yoshikawa, K. Kobayashi, Y. Imakura, K.S. Im, and Y. Ikenishi, *Chem. Pharm. Bull.*, **28**, 296 (1980).
2) Location: *133-1, Yamada-kami, Suita, Osaka 565, Japan.*
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4) I. Kitagawa, K.S. Im, and Y. Fujimoto, *Chem. Pharm. Bull.*, **25**, 800 (1977).
5) *a*) I. Kitagawa, M. Yoshikawa, Y. Imakura, and I. Yosioka, *Chem. and Ind.*, **1973**, 276; *b*) I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Tetrahedron Lett.*, **1973**, 3997; *c*) I. Kitagawa, M. Yoshikawa, Y. Imakura, and I. Yosioka, *Chem. Pharm. Bull.*, **22**, 1339 (1974).
6) *a*) I. Kitagawa, M. Yoshikawa, Y. Ikenishi, K.S. Im, and I. Yosioka, *Tetrahedron Lett.*, **1976**, 549; *b*) I. Kitagawa, M. Yoshikawa, K.S. Im, and Y. Ikenishi, *Chem. Pharm. Bull.*, **25**, 657 (1977); *c*) I. Kitagawa, M. Yoshikawa, and A. Kadota, *ibid.*, **26**, 484 (1978).
7) I. Kitagawa, Y. Ikenishi, M. Yoshikawa, and K.S. Im., *Chem. Pharm. Bull.*, **25**, 1408 (1977).
8) *a*) Presented at the Annual Meeting of the Pharmaceutical Society of Japan by I. Kitagawa, M. Yoshikawa, and H. Ohmori (Tokyo, April, 1977), Abstract Paper II-219; *b*) A partial preliminary account has appeared (ref. 3).
9) *a*) S. Torii, *Yuki Gosei Kagaku Kyokai Shi*, **32**, 161 (1974); *b*) S. Torii, T. Okamoto, G. Tanida, H. Hino, and Y. Kitsuya, *J. Org. Chem.*, **41**, 166 (1976).

However, no work has been reported on the anodic oxidation of uronic acid derivatives, while only a few investigations dealing with the anodic oxidation of acetal functions of carbohydrates and the electrolytic degradation of carbohydrate derivatives have appeared.[10]

We started our investigation on the electrolytic decarboxylation reaction of uronic acids with methyl glucuronide derivatives. First, the oxidation potential of methyl 2,3,4-tri-O-methyl-β-D-glucopyranosiduronic acid (**1**)[6c] was measured by cyclic voltammetry in acetonitrile containing ethyl tri-*n*-butylammonium tetrafluoroborate and shown to be 2.10 V *vs* a standard calomel electrode (S.C.E.), whereas that of the sodium salt (**1a**) was shown to be 1.76 V *vs* S.C.E. Thus, the sodium salt (**1a**) was subjected to controlled potential electrolysis at 1.72 V *vs* S.C.E. in methanol containing sodium perchlorate to furnish two decarboxylation products: **2** (63%) and **3** (33%).

The major product (**2**) lacks the carboxylic function, as shown by its infrared (IR) spectrum. The proton magnetic resonance (^{1}H-NMR) spectrum, which exhibits signals due to five methoxyl groups, and the mass spectrum, which gives fragment ions **i**, **ii**, and **iii**[11] along with the molecular ion at *m/z* 236, suggest replacement of the carboxylate moiety in **1a** with a methoxyl group. Furthermore, two acetal proton signals at δ 4.47 (1H, d, J=7 Hz) and δ 4.80 (1H, d, J=3.5 Hz), which are assignable to C_1–H and C_5–H respectively, substantiate the structure **2**, having a newly introduced axial 5α-methoxyl group, for the major product.

Spectral analysis in comparison with **2** showed the minor product (**3**) to be an isomer of the major one. Since the ^{1}H-NMR spectrum of **3** taken in deuterochloroform exhibits a two-proton doublet-like signal due to C_1–H and C_5–H at δ 4.41 (J=*ca.* 7 Hz), the structure **3** with an equatorial 5β-methoxyl group has been assigned. The rather broadened doublet is probably due to the virtual longrange coupling,[6b] as revealed by ^{1}H-NMR spectra of **3** taken in some other solvents (see "Experimental").

When methyl glucuronide (**1**) was subjected to constant current electrolysis for 2 hour (glassy carbon electrode; 0.16 A) in methanol containing diethylamine, the same two isomers, **2** (37%) and **3** (32%), were obtained. On the other hand, when constant current electrolysis (Pt electrode; 0.1 A) of **1** was carried out in acetic acid containing triethylamine for 3 hour, two C_5-acetoxylated products, **4** (35%)[6c] and **5** (34%),[6c] were obtained.

It has been clarified so far that the carboxyl moiety of glucuronic acid is readily converted in good yield either to a methoxyl or to an acetoxyl moiety under anodic oxidation conditions. Since both methoxyl and acetoxyl derivatives thus formed are suitable intermediates for splitting original glycosidic linkages under mild reaction conditions,[6] the electrolytic decarboxylation reaction represents a promising initial reaction for cleavage of the glucuronide linkage as in the case of the lead tetraacetate degradation.[6] Thus, application of the reaction has been investigated using several glucuronide-saponins[3] as substrates.

Electrolytic Degradation of Sakuraso-saponin (6)

When sakuraso-saponin (**6**)[1] was oxidized in acetic acid containing triethylamine under constant current electrolysis conditions as for **1** and the whole reaction mixture was subsequently treated with 1% sodium methoxide in methanol, the starting glucuronide-saponin was found to be decomposed to afford the sapogenol and carbohydrate moieties. The reaction products were acetylated to facilitate isolation and characterized as 3,16-di-O-acetyl-protoprimulagenin A (**7a**, 21%)[7] and 3-O-acetyl-aegicerin (**8a**, 27%),[12] both derived from the sapogenol moiety of **6**, together with 1,2,3,4,6-penta-O-acetyl-D-glucopyranose (**9a**, 48%)[7] and a trisaccharide nonaacetate (**10a**, 35%),[7] derived from the oligosaccharide moiety of **6**. Although no compound originating from the glucuronic acid moiety of **6** was obtained, the

10) *a*) N.L. Weinberg and H.R. Weinberg, *Chem. Rev.*, **68**, 449 (1968); *b*) M. Fedoronko, *Advan. Carbohyd. Chem. and Biochem.*, **29**, 107 (1974).
11) J. Lönngren and S. Svensson, *Advan. Carbohyd. Chem. and Biochem.*, **29**, 41 (1974).
12) I. Kitagawa, A. Matsuda, and I. Yosioka, *Chem. Pharm. Bull.*, **20**, 2226 (1972).

anodic oxidation has been shown useful for selective cleavage of the glucuronide linkage in a glucuronide-saponin, as was the case with the lead tetraacetate degradation.[6] It should be noted here that previous protection of hydroxyl functions in the starting glucuronide-saponin is unnecessary in this electrochemical degradation. However, as noted above in the formation of 3-O-acetyl-aegicerin (**8a**), the anodic oxidation was accompanied by concomitant oxidation of the 16α-hydroxyl group in the sapogenol moiety. In order to throw light upon this matter, protoprimulagenin A (**7**) was subjected to constant current electrolysis as described for sakuraso-saponin (**6**), and it was found that protoprimulagenin A (**7**) was readily converted to aegicerin (**8**)[12] in 65% yield *via* selective oxidation of its axial 16α-hydroxyl group. Next, in order to avoid such concomitant oxidation of the axial hydroxyl function in the sapogenol

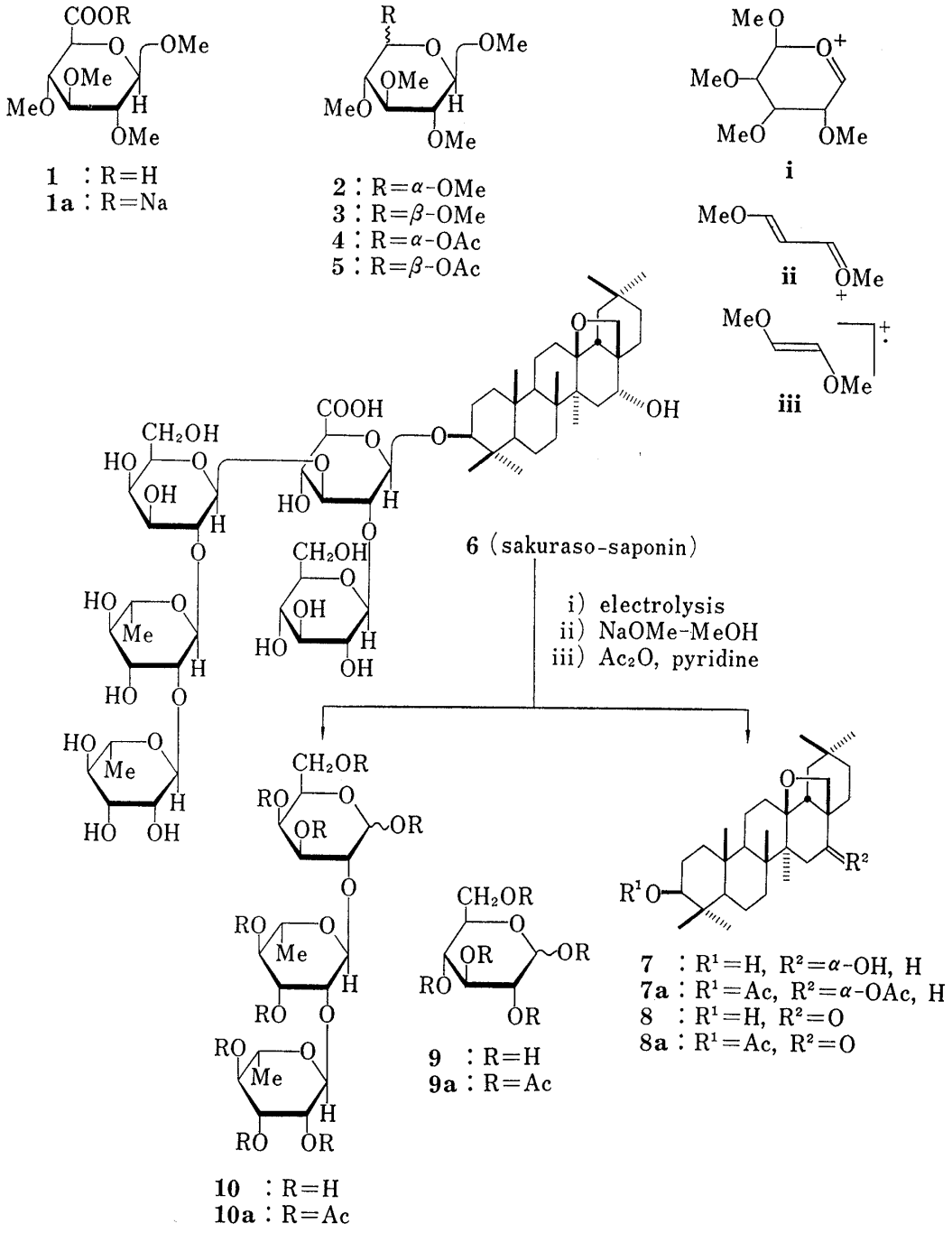
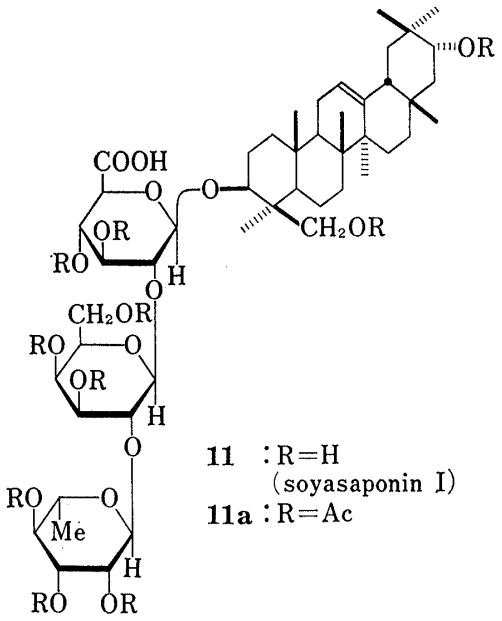


Chart 1

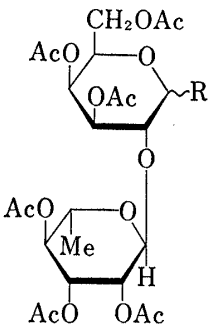
moiety of saponin, the anodic oxidations of soyasaponin I (**11**)[13] and desacyl-jegosaponin (**15**)[1] were carried out using their acetylated derivatives.

Electrolytic Degradation of Soyasaponin I (11) and Desacyl-jegosaponin (15)

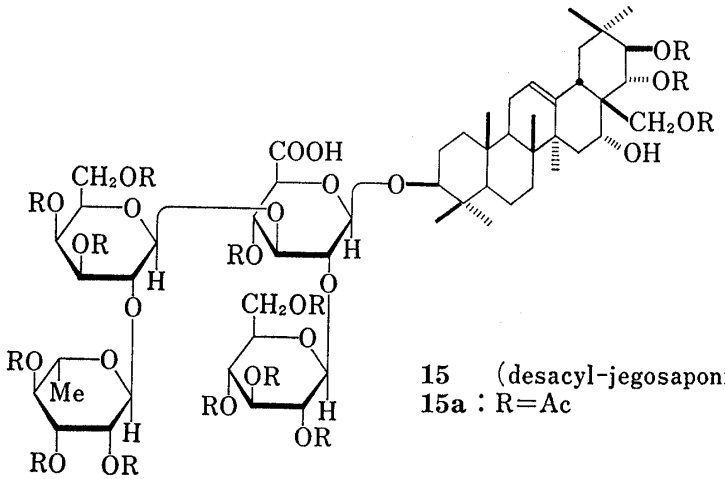
A deca-O-acetyl derivative (**11a**)[7] of soyasaponin I (**11**) was subjected to anodic oxidation followed by alkali treatment and acetylation as described for sakuraso-saponin (**6**). In this case, however, two disaccharide heptaacetates (**13**, 26%; **14**, 23%)[7] were the only separable products derived from the carbohydrate moiety of **11a**, and the sapogenol moiety gave a complex mixture of products from which no pure substance could be isolated. In the case of desacyl-jegosaponin (**15**),[1] since the fully acetylated derivative could not be prepared due to degradation under the acetylation conditions (heating with acetic anhydride and pyridine),[7] the tetradecaacetate (**15a**) was subjected to the electrolytic degradation, although **15a** possessed an axial 16α-hydroxyl group which was expected to be unfavorable for anodic oxidation



11 : R=H (soyasaponin I)
11a : R=Ac



13 : R=α-OAc
14 : R=β-OAc



15 (desacyl-jegosaponin) : R=H
15a : R=Ac

Chart 2

13) I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Chem. Pharm. Bull.*, **24**, 121 (1976).

(*vide supra*). The products were 1,2,3,4,6-penta-O-acetyl-D-glucopyranose (**9a**, 38%)[7] and the two disaccharide heptaacetates (**13**, 26%; **14**, 24%) mentioned above, all of which were derived from the oligosaccharide moiety of **15a**. Here again, no separable product deriving from the sapogenol moiety was detected in the reaction mixture.

These results led us to suspect that some unexpected side reactions may occur in the sapogenol moiety during anodic oxidation. In order to shed light upon the matter, we next examined the behavior of Δ^{12}-oleanene-type sapogenol in anodic oxidation.

Electrolysis of Soyasapogenol B Triacetate (16)

When 3,21,24-tri-O-acetyl-soyasapogenol B (**16**)[13] was subjected to constant current electrolysis (0.45 A) in acetic acid containing triethylamine as described for **11a** and **15a** for a long period (12 hr), a complex mixture of products was formed, as in the case of the anodic oxidation of **11a**. However, when the anodic oxidation was carried out under lower current conditions (0.05 A) for a shorter reaction period (1 hr), two triterpenic products were obtained in good yields: **17** (5%) and **18** (75%).

The IR spectrum of the minor product (**17**) shows a strong acetoxyl absorption band whereas the ultraviolet (UV) spectrum suggests the presence of a heteroannular diene moiety in **17** in view of the characteristic triplet absorption maxima[14] at 243 nm (ε 30000), 251 (34000), and 261 (22000). The ^{1}H-NMR spectrum of **17** shows two adjacent olefinic proton signals (C_{11}–H, C_{12}–H) at δ 5.52 (1H, d, J=11 Hz) and δ 6.33 (1H, br. d, J=11 Hz), together with three acetoxyl signals. Based on these findings, the minor product has been assigned the structure **17**. The assignment was corroborated by direct comparison with the authentic compound (**17**) prepared by selenium oxide oxidation[14] of **16**.

The major product (**18**) is a tetra-O-acetyl derivative, as shown by its strong acetoxyl absorption band in the IR spectrum and four acetoxyl signals in the ^{1}H-NMR spectrum. Further, the ^{1}H-NMR spectrum shows signals at δ 5.17 (1H, d, J=3.5 Hz) due to C_{12}–H and at δ 5.36 (1H, d.d, J=3.5, 8 Hz) due to C_{11}–H. When **18** was treated with mild acid, the above diene (**17**) was readily formed. Based on these findings, the structure **18** was assigned to the major product. The assignment was further supported by identification of the product with the authentic allyl acetate (**18**), which was synthesized by acetoxylation of **16** with N-bromosuccinimide and lead tetraacetate.[15]

As has been elucidated in the anodic oxidation of **16**, Δ^{12}-oleanene-type sapogenols may be readily oxidized at their allylic C-11 positions and this property may be a principal reason for the formation of complicated reaction products in the anodic oxidations of acetylated derivatives of soyasaponin I (**11**) and desacyl-jegosaponin (**15**).

Electrolysis of Glycyrrhizin Monomethyl Ester (19b)

We next examined the anodic oxidation of another readily available glucuronide-saponin glycyrrhizin (**19**), which possesses an intrinsic oxidized structure at C-11 in the sapogenol moiety. In this case, a monomethyl ester (**19b**), in which only the carboxyl group in the sapogenol moiety was methylated, was chosen as the starting material.

Methylation of glycyrrhizin (**19**) with diazomethane gave a trimethyl ester (**19a**),[16] which, on mild alkali treatment, was converted to a monomethyl ester (**19b**). The IR spectrum of **19b** shows the presence of the ester function, while the ^{1}H-NMR spectrum (in d_5-pyridine) shows a three-proton singlet at δ 3.71 due to one methoxycarbonyl group. Hydrolysis of **19b** with 20% aqueous sulfuric acid in dioxane afforded methyl glycyrrhetinate (**20**), thus confirming the structure of **19b**.

14) S. Shibata, I. Kitagawa, and H. Fujimoto, *Chem. Pharm. Bull.*, **14**, 1023 (1966).
15) *a*) D.H.R. Barton, E.F. Lier, and J.F. McGhie, *J. Chem. Soc.* (*C*), **1968**, 1031; *b*) I. Kitagawa, K. Kitazawa, and I. Yosioka, *Tetrahedron*, **28**, 907 (1972).
16) C.H. Brieskorn and H. Sax, *Arch. Pharm.*, **303**, 905 (1970).

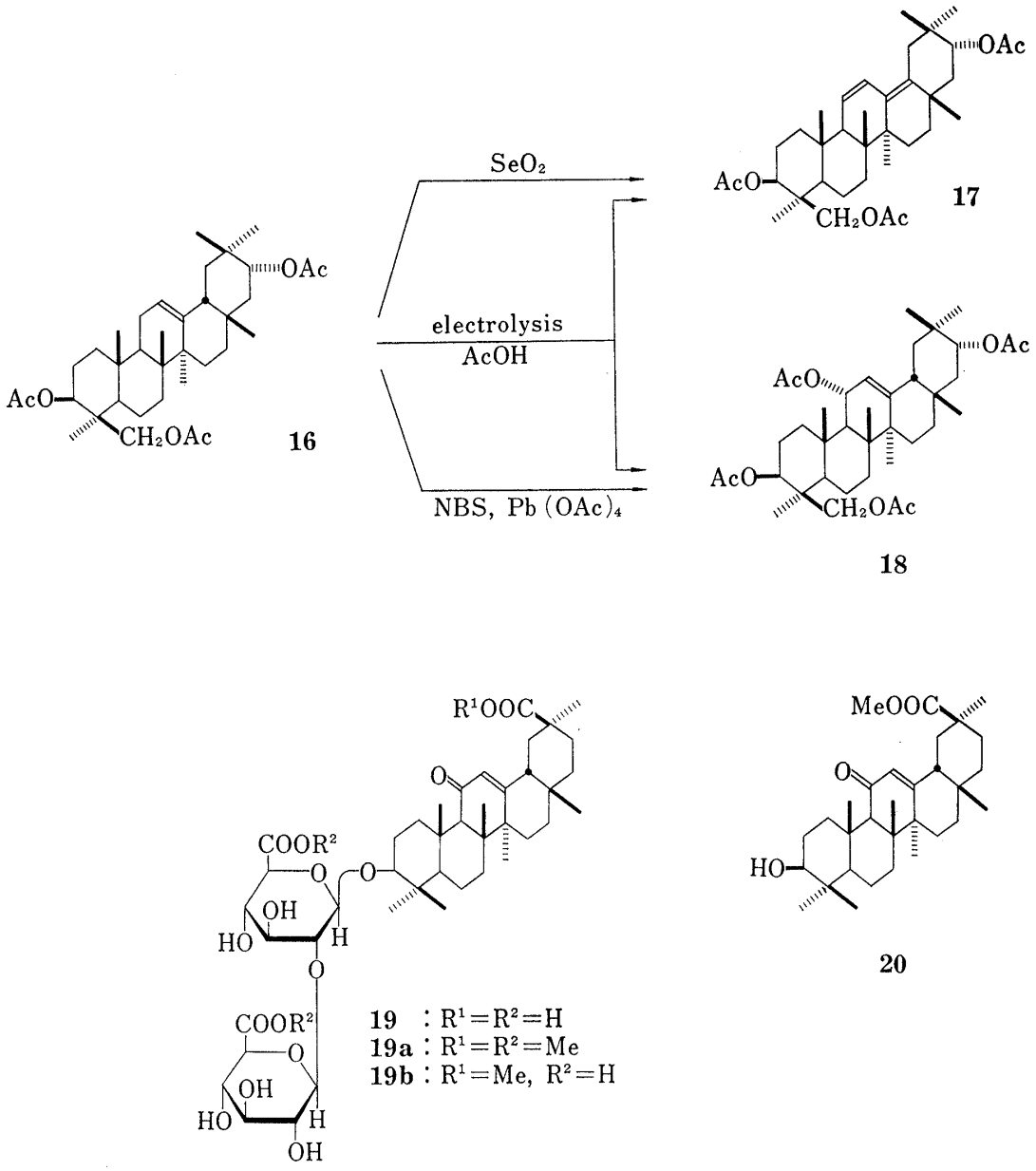


Chart 3

When the monomethyl ester (**19b**) was subjected to anodic oxidation followed by alkali treatment, methyl glycyrrhetinate (**20**) was obtained in 30% yield, but no product deriving from the glucuronic acid moiety was obtained. It should be noted here that an unidentified sapogenol was isolated in 20% yield in this case, and the structure is currently under study.

It has been shown that the electrolytic decarboxylation reaction is useful as an initial step in the selective cleavage of the glucuronide linkage in saponins, in the same way as lead tetraacetate degradation.[6] The present electrochemical method is advantageous as compared with the lead tetraacetate degradation method[6] in the sense that it does not require protection of hydroxyl functions in the carbohydrate moiety of the starting glucuronide-saponin. Although no product originating from the glucuronic acid moiety has yet been characterized, isolation and structure elucidation of the products are currently under way. Furthermore, the selective oxidation of an axial hydroxyl group and allylic oxidation mentioned above are currently under further study.

Experimental[17]

Cyclic Voltammetry of 1 and 1a——a) A solution of **1** (50 mg) in acetonitrile (20 ml)[19] was supplemented with ethyl tri-*n*-butylammonium tetrafluoroborate (600 mg)[20] and the cyclic voltammetry was carried out at 25° to a peak potential of 2.10 V *vs.* S.C.E.

b) A solution of **1** (100 mg) in MeOH (10 ml) was neutralized with 5% NaOMe–MeOH. Removal of MeOH under reduced pressure gave **1a** (110 mg) as a colorless oil. IR ν_{max}^{Nujol} cm^{-1}: 1610, 1420, 1080. Cyclic voltammetry of **1a** (50 mg) in MeOH (20 ml) containing sodium perchlorate (245 mg)[21] was carried out at 25° to a peak potential of 1.76 V *vs.* S.C.E.

Controlled Potential Electrolysis of 1a——Sodium perchlorate (500 mg) was added to a solution of **1a** (150 mg) in MeOH (50 ml) and the whole solution in an open beaker was subjected to controlled potential electrolysis at 1.72 V *vs.* S.C.E. at 25° with stirring.[22] After removal of MeOH under reduced pressure, the reaction product was treated with water and extracted with AcOEt. The AcOEt extract, after drying with $MgSO_4$, was concentrated under reduced pressure to give a syrup which was purified by preparative TLC (*n*-hexane–AcOEt=1: 1) to furnish **2** (91 mg, 63%) and **3** (47 mg, 33%). **2**, colorless oil, $[\alpha]_D^{28}$ −109.7° (c=10.2, $CHCl_3$). High resolution MS (*m/z*): Calcd for $C_{10}H_{20}O_6$ (M^+) 236.126, $C_9H_{17}O_5$ (**i**) 205.108, $C_5H_9O_2$ (**ii**) 101.060, $C_4H_8O_2$ (**iii**) 88.052. Found: 236.125, 205.110, 101.061, 88.055. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2930, 1093. ^{1}H-NMR ($CDCl_3$, δ): 3.49 (3H, s), 3.51 (6H, s), 3.56, 3.60 (3H each, both s) (OMe×5), 4.47 (1H, d, J=7 Hz, 1-H), 4.80 (1H, d, J=3.5 Hz, 5-H). **3**, mp 55—55.5° (colorless needles from CCl_4), $[\alpha]_D^{28}$ 0° (c=5.0, $CHCl_3$). High resolution MS (*m/z*): Calcd for $C_{10}H_{20}O_6$ (M^+) 236.126, $C_9H_{17}O_5$ (**i**) 205.108, $C_5H_9O_2$ (**ii**) 101.060, $C_4H_8O_2$ (**iii**) 88.052. Found: 236.127, 205.110, 101.061, 88.055. ^{1}H-NMR (δ): (in $CDCl_3$) 3.51, 3.53 (6H each, both s), 3.57 (3H, s) (OMe×5), 4.41 (2H, m, signal width=7 Hz, 1-H, 5-H); (in CCl_4) 3.43, 3.45 (6H each, both s), 3.49 (3H, s), (OMe×5), 4.17 (2H, d-like, signal width=7 Hz, 1-H, 5-H); (in d_6-acetone): 3.46 (12H, s), 3.49 (3H, s) (OMe×5), 4.31 (2H, d-like, signal width=7 Hz, 1-H, 5-H); (in d_6-benzene) 3.33, 3.49 (6H each, both s), 3.56 (3H, s) (OMe×5), 4.27 (2H, m, signal width=7 Hz, 1-H, 5-H).

Constant Current Electrolysis of 1——a) A solution of **1** (200 mg) in MeOH (50 ml) containing Et_2NH (0.2 ml) was subjected to constant current electrolysis for 2 hr [Pt electrode; 160 mA (4 mA/cm^2); 28—40 V; 24°]. The product obtained upon removal of MeOH under reduced pressure was purified by preparative TLC (*n*-hexane–AcOEt=1: 1) to furnish **2** (88 mg, 37%) and **3** (75 mg, 32%).

b) A solution of **1** (1 g) in MeOH (100 ml) containing Et_2NH (0.5 ml) was subjected to constant current electrolysis [glassy carbon; 400 mA (16 mA/cm^2); 50—60 V] at 24° for 8 hr. The product obtained upon removal of the solvent under reduced pressure was purified by column chromatography (SiO_2 100 g, *n*-hexane-AcOEt=50: 1—20: 1) to furnish **2** (360 mg) and **3** (310 mg). **2** and **3** obtained here by constant current electrolysis of **1** were identical with those obtained above by controlled potential electrolysis of **1a** as determined by TLC (*n*-hexane–AcOEt=1: 2, *n*-hexane–ether=1: 2, benzene–MeOH=40: 1, benzene–acetone=10: 1) and IR spectroscopy (CCl_4).

Electrolytic Acetoxylation of 1——A solution of **1** (100 mg) in AcOH (20 ml) containing Et_3N (0.5 ml) was subjected to constant current electrolysis [Pt; 100 mA (5 mA/cm^2); 50—60 V] at 5—7° for 3 hr. The reaction mixture was poured into cold water and neutralized with $NaHCO_3$ (powder). The whole mixture was then extracted with AcOEt. After the usual work-up and removal of the solvent under reduced pressure, the extract gave a syrup which was purified by preparative TLC (*n*-hexane–AcOEt=2: 1) to furnish **4** (37 mg) and **5** (36 mg). **4** and **5** were identical with the authentic samples[6c] as judged by TLC (*n*-hexane–AcOEt=1: 1, benzene–acetone=10: 1, benzene–MeOH=40: 1), GLC [15% neopentyl glycol succinate on Uniport B (80—100 mesh), 3 mm×2 m, N_2 flow rate 35 ml/min, column temp. 175°), IR (CCl_4), and ^{1}H-NMR (CCl_4).

Electrolysis of Sakuraso-saponin (6)——A solution of **6** (500 mg) in AcOH (25 ml) containing Et_3N (1.2

17) The instruments used to obtain physical data, and the experimental conditions for chromatography were the same as in our recent paper,[18] unless otherwise specified. Cyclic voltammetry was carried out in a three-electrode system using a linear scanning unit (Hokuto Denko Co., model HB-101) connected with a potentiostat (Hokuto Denko Co., model PS-500B). As electrode systems, a glassy-carbon indicator electrode, a glassy-carbon counter electrode, and a saturated calomel electrode (S.C.E.) were used. The indicator electrode was prepared from cut glassy carbon (GC-20, Tokai Carbon Co.) coated on the side with epoxy-resin, in a glass cylinder. The S.C.E. was separated by an agar plug containing 0.1 M sodium perchlorate. Current-potential curves were recorded on a Toa XYR-2A XY recorder at a scanning rate of 50 mV sec^{-1}. For preparative oxidations, a potentiostat/galvanostat apparatus (Hokuto Denko Co., model HA-105) was used.

18) I. Kitagawa, Y. Hamamoto, and M. Kobayashi, *Chem. Pharm. Bull.*, **27**, 1934 (1979).

19) Acetonitrile was purified by the reported method: J.F. O'Donnell, J.T. Ayres, and C.K. Mann, *Anal. Chem.*, **37**, 1161 (1965).

20) H.O. House, E. Feng, and N.P. Peet, *J. Org. Chem.*, **36**, 2371 (1971).

21) Purified by recrystallization from aq. EtOH followed by drying *in vacuo*.

22) All the following preparative electrolysis experiments were carried out in open beakers with stirring.

ml) was subjected to constant current electrolysis [Pt; 240 mA (10 mA/cm²); 30—50 V] at 5—7° for 8 hr. After pouring into ice-water and neutralization with $NaHCO_3$ (powder), the whole reaction mixture was extracted with *n*-BuOH. After the usual work-up, the *n*-BuOH was removed under reduced pressure to give the residue which was mixed with silica gel (5 g) with the aid of a small amount of MeOH. The mixture was dried with a heat lamp. The silica gel mixture was then put on a column of silica gel (50 g) and eluted with $CHCl_3$–MeOH–H_2O=65: 35: 10 (lower phase) to furnish the oxidation product (150 mg) and unchanged **6** (200 mg). The oxidation product, without further purification, was dissolved in 1% NaOMe–MeOH (10 ml) and stirred at 24° for 30 min. The whole mixture was then neutralized with Dowex 50 W×8 (H^+ form, 20 ml) and filtered. The filtrate was concentrated under reduced pressure and dried to give the residue, which was acetylated with Ac_2O–pyridine (1: 1, 5 ml) at 35° with stirring for 24 hr. The reaction mixture was poured into ice-water and extracted with AcOEt. After the usual work-up, the product obtained from the AcOEt extract was purified by preparative TLC (benzene–acetone=3: 1) to furnish 3,16-di-O-acetyl-protoprimulagenin A (**7a**, 21 mg, 21%), 3-O-acetyl-aegicerin (**8a**, 25 mg, 27%), 1,2,3,4,6-penta-O-acetyl-D-glucopyranose (**9a**, 43 mg, 48%), and a trisaccharide nonaacetate (**10a**, 71 mg, 36%). **7a** and **8a** were identical with corresponding authentic samples[7,11] as judged by IR (CCl_4) and TLC ($CHCl_3$–MeOH=50: 1, *n*-hexane–ether=2: 1, benzene–acetone=20: 1). **9a** and **10a** were identical with corresponding authentic samples[7] as judged by TLC (benzene–acetone=5: 1, *n*-hexane–ether=1: 2, benzene–MeOH=20: 1), IR (CCl_4), and ^{1}H-NMR ($CDCl_3$).

A solution of **9a** or **10a** (10 mg each) in 1% NaOMe–MeOH (1 ml) was stirred for one hour at room temp. and neutralized with Dowex 50 W×8 (H^+ form, 5 ml). The filtrate was concentrated under reduced pressure to give the residue (**9** or **10**) which was identical with an authentic sample[7] as judged by TLC ($CHCl_3$–MeOH=1: 1, $CHCl_3$–MeOH–H_2O=6: 4: 1, *n*-BuOH–EtOH–H_2O=5: 3: 2, CH_3CN–H_2O=4: 1) and PPC (iso-PrOH–*n*-BuOH–H_2O=7: 1: 2, *n*-BuOH–pyridine–H_2O=6: 4: 3).

Electrolytic Oxidation of Protoprimulagenin A (**7**)——A solution of **7** (150 mg) in AcOH (20 ml) containing Et_3N (0.5 ml) was subjected to constant current electrolysis [Pt; 100 mA (8 mA/cm²); 45—50 V] at 5—7° for 12 hr. After treating the reaction mixture as described for the electrolytic acetoxylation of **1**, the product obtained by extraction with AcOEt was purified by preparative TLC ($CHCl_3$–MeOH=30: 1) to furnish aegicerin (**8**, 65 mg), which was identical with an authentic sample[12] as judged by IR (KBr) and TLC ($CHCl_3$–MeOH=60: 1, benzene–acetone=3: 1, benzene–MeOH=10: 1), together with recovered **7** (50 mg).

Electrolysis of Soyasaponin I Deca-O-acetate (**11a**)——The deca-O-acetate **11a** (510 mg) was prepared by acetylation of soyasaponin I (**11**) with Ac_2O–pyridine (1: 1, 20 ml) at 15° for 12 hr. The deca-O-acetate (**11a**, 100 mg) was dissolved in AcOH (12 ml) containing Et_3N (1.2 ml) and subjected to constant current electrolysis [Pt; 250 mA (20 mA/cm²); 40—60 V] at 5—7° for 3 hr. The reaction mixture was worked up as described for **6** and the *n*-BuOH extract was concentrated under reduced pressure. The residue thus obtained was dissolved in 5% NaOMe–MeOH (5 ml) and the solution was stirred at 15° for 3 hr and neutralized with Dowex 50 W×8 (H^+ form). After removing the resin by filtration, the filtrate was concentrated under reduced pressure. The residue was dried *in vacuo* and acetylated with Ac_2O–pyridine (1: 1, 2 ml) at 36° for 12 hr. The reaction mixture was poured into ice-water and extracted with ether. The usual work-up of the extract gave a product which was purified by preparative TLC (benzene–acetone=7: 1) to furnish a mixture of disaccharide heptaacetates (32 mg). Repeated preparative TLC purification (*n*-hexane–ether=1: 6) of the mixture gave the α-anomer (**13**, 11 mg, 26%) and the β-anomer (**14**, 10 mg, 23%), which were identical with corresponding authentic samples[7] as judged by IR (CCl_4) and TLC (*n*-hexane–ether=1: 3, benzene–MeOH=20: 1, benzene–acetone=20: 1).

Electrolysis of Desacyl-jegosaponin Hexadeca-O-acetate (**15a**)——The hexadeca-O-acetate **15a** (250 mg) was prepared by acetylation of desacyl-jegosaponin (**15**) as described for the acetylation of soyasaponin I (**11**). **15a**, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460, 1741, 1220, ^{1}H-NMR ($CDCl_3$, δ): 0.82 (3H), 0.92 (9H), 1.01, 1.06, 1.26 (3H each) (all s, *tert.* CH_3×7), 1.98—2.16 (42H, OAc×14). A solution of **15a** (250 mg) in AcOH (15 ml) containing Et_3N (1.5 ml) was subjected to constant current electrolysis [Pt; 450 mA (40 mA/cm²); 35—60 V] at 5—7° for 8 hr. The reaction mixture was poured into ice-water and the precipitate (240 mg) was collected by filtration then dissolved in 5% NaOMe–MeOH (2 ml), and the solution was stirred at 15° for one hour. After neutralizing the reaction mixture as described in the case of **11**, the product was acetylated with Ac_2O–pyridine (1: 1, 5 ml) and worked up as usual. The product obtained by extraction with AcOEt was purified by preparative TLC (benzene–acetone=8: 1) to furnish a mixture of disaccharide heptaacetates (**13**, **14**, 70 mg) and 1,2,3,4,6-penta-O-acetyl-D-glucopyranose (**9a**, 30 mg, 38%). The former mixture was further purified by preparative TLC (*n*-hexane–ether=1: 6) to furnish **13** (32 mg, 26%) and **14** (30 mg, 24%). **9a**, **13**, **14** were identified as described above.

Electrolysis of Soyasapogenol B Triacetate (**16**)——A solution of 3,21,24-tri-O-acetyl-soyasapogenol B (**16**, 100 mg) in AcOH (20 ml) containing Et_3N (1 ml) was subjected to constant current electrolysis [Pt; 50 mA (2 mA/cm²); 20—30 V] at 5° for one hour. The reaction mixture was poured into ice-water and the precipitate was collected by filtration and purified by preparative TLC (*n*-hexane–ether=1: 2) to furnish **17** (5 mg, 5%) and **18** (76 mg, 75%). **17**, mp 251—252° (colorless needles from $CHCl_3$–MeOH), $[\alpha]_D^{25}$ −24.6° (*c*=0.83, CCl_4). *Anal.* Calcd for $C_{36}H_{54}O_6$: C, 74.19; H, 9.34. Found: C, 73.94; H, 9.27. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2950, 1739, 1238. UV λ_{max}^{EtOH} nm (ε): 243 (30000), 251 (34000), 261 (22000). ^{1}H-NMR (CCl_4, δ): 0.70, 0.89 (3H each,

both s), 0.95 (6H, s), 0.97, 0.99, 1.06 (3H each, all s) (*tert.* $CH_3 \times 7$), 1.98 (9H, s, OAc × 3), 4.01, 4.21 (2H, ABq, $J=11$ Hz, $-C\underline{H}_2OAc$), 4.49 (2H, m, 3-H, 21-H), 5.52 (1H, d, $J=11$ Hz), 6.33 (1H, br.d, $J=ca.$ 11 Hz) (11,12-H_2). MS (*m/z*, %): 582 (M^+, 100), 522 (37), 507 (26), 201 (100). **18**, white powder, $[\alpha]_D^{25}$ +1.0° ($c=1.0$, CCl_4). *Anal.* Calcd for $C_{38}H_{58}O_8$: C, 70.99; H, 9.09. Found: C, 70.75; H, 9.05. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2960, 1750, 1745, 1235. ^{1}H-NMR (CCl_4, δ): 0.79, 0.92 (3H each, both s), 1.01 (6H, s), 1.06, 1.09, 1.23 (3H each, all s) (*tert.* $CH_3 \times 7$), 1.96 (3H, s), 1.98 (9H, s), (OAc × 4), 4.12, 4.33 (2H, ABq, $J=11$ Hz, $-C\underline{H}_2OAc$), 4.58 (2H, m, 3-H, 21-H), 5.17 (1H, d, $J=3.5$ Hz, 12-H), 5.36 (1H, d.d, $J=3.5$, 8 Hz, 11-H). MS (*m/z*, %): 642 (M^+, 2), 582 (100), 522 (13), 507 (6), 201 (100).

Selenium Oxide Oxidation of 16——A solution of **16** (30 mg) in AcOH (3 ml) was treated with SeO_2 (30 mg) and the whole mixture was heated under reflux for one hour. The reaction mixture was then poured into cold water and the precipitate was collected by filtration and crystallized from $CHCl_3$–MeOH to furnish a dienic compound (27 mg, colorless needles) which was identical with **17** as judged by mixed mp determination (251—252°), TLC (benzene–acetone=10: 1, benzene–MeOH=40: 1, $CHCl_3$, *n*-hexane–ether=1: 2), and IR spectroscopy (CCl_4).

Acid Treatment of 18 giving 17——A solution of **18** (30 mg) in MeOH (5 ml) was treated with 5% HCl–dry MeOH (0.1 ml) and stirred at 15° for 3 hr. The whole reaction mixture was diluted with water. The precipitate was collected by filtration and crystallized from $CHCl_3$–MeOH to furnish **17** (20 mg, colorless needles) which was identical with an authentic sample as judged by mixed mp determination, TLC, and IR as described above.

N-Bromosuccinimide-Lead Tetraacetate Oxidation of 16 giving 18——A solution of **16** (40 mg) in dry benzene (5 ml) was treated with NBS (40 mg) and $Pb(OAc)_4$ (80 mg) and the whole mixture was heated under reflux for 30 min. After dilution with ether, the whole mixture was washed successively with aq. $NaHCO_3$ and water. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to give a syrup which was solidified with acetone and water. The white solid (43 mg) thus obtained was identified as **18** by TLC (benzene–MeOH=40: 1, *n*-hexane–ether=1: 1, benzene–acetone=10: 1, $CHCl_3$) and IR spectroscopy (CCl_4).

Glycyrrhizin Monomethyl Ester (19b)——A solution of crude glycyrrhizin (20 g, isolated from Glycyrrhizae Radix) in MeOH (100 ml) was treated with ethereal diazomethane and the whole solution was allowed to stand at 25° for 12 hr. The residue, obtained after removal of the solvent under reduced pressure, was purified by column chromatography (SiO_2 1 kg, $CHCl_3$–MeOH=50: 1—10: 1) and by crystallization from $CHCl_3$–MeOH to furnish glycyrrhizin trimethyl ester (**19a**, 5.2 g) as colorless needles of mp 283—285°, $[\alpha]_D^{26}$ +48.4° ($c=0.6$, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3450, 1763, 1735, 1664, 1065 [lit.:[16] mp 283—286° (from MeOH–H_2O), $[\alpha]_D^{20}$ +46.7° ($c=0.64$, EtOH), IR ν_{max}^{KBr} cm^{-1}: 3440—3500, 1725—1750, 1615—1655]. A solution of **19a** (2 g) in MeOH (100 ml) was treated with 5% aq. KOH (25 ml) and the whole solution was stirred at 25° for 3 hr. After neutralization with Dowex 50 W × 8 (H^+ form), the filtrate was concentrated under reduced pressure to give a white powder (1.95 g). Crystallization of the powder from MeOH furnished glycyrrhizin monomethyl ester (**19b**) as colorless fine crystals of mp 190—191°, $[\alpha]_D^{22}$ +68.6° ($c=1.0$, MeOH). *Anal.* Calcd for $C_{43}H_{64}O_6 \cdot 2H_2O$: C, 59.15; H, 7.85. Found: C, 58.83; H, 7.48. IR ν_{max}^{KBr} cm^{-1}: 3400, 1735, 1660, 1050. ^{1}H-NMR (d_5-pyridine, δ): 0.70, 1.04, 1.10, 1.15, 1.18, 1.31, 1.39 (3H each, all s, *tert.* $CH_3 \times 7$), 3.71 (3H, s, OMe), 4.90, 5.22 (1H each, both d, $J=6.5$ Hz, anomeric H × 2), 5.78 (1H, s, 12-H).

Acid Hydrolysis of 19b giving 20——A solution of **19b** (30 mg) in 20% aq. H_2SO_4–dioxane (1: 1, 2 ml) was heated under reflux for 2 hr. After cooling, the reaction mixture was treated with ice-water. The precipitate (18 mg) was collected by filtration and purified by preparative TLC (benzene–acetone=2: 1) to furnish **20** (6 mg) which was identical with methyl glycyrrhetinate as judged by TLC (benzene–acetone=5: 1, $CHCl_3$–MeOH=30: 1, benzene–MeOH=10: 1) and IR (KBr).

Electrolysis of Glycyrrizin Monomethyl Ester (19b)——A solution of **19a** (500 mg) in AcOH (50 ml) containing Et_3N (1 ml) was subjected to constant current electrolysis [Pt; 500 mA (10 mA/cm^2); 40—60 V] at 5—7° for 24 hr. After working up the reaction mixture as described for the electrolysis of **11a**, the *n*-BuOH extract was treated with 5% NaOMe–MeOH (20 ml). The whole mixture was neutralized with Dowex 50 W × 8 (H^+ form) and the solvent was removed under reduced pressure. The residue was then purified by preparative TLC (benzene–acetone=3: 1) to furnish **20** (84 mg) and an unidentified sapogenol (56 mg). **20** was identified as methyl glycyrrhetinate by TLC (as above), IR (KBr), and ^{1}H-NMR ($CDCl_3$). Unidentified sapogenol, mp 224—226° (colorless needles from $CHCl_3$–MeOH), $[\alpha]_D^{20}$ +99° ($c=1.0$, MeOH). High resolution MS (*m/z*): Calcd for $C_{31}H_{46}O_4$ (M^+) 482.339; Found: 482.337. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610, 3450, 2952, 1733, 1661, 1630.

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