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Terpenoids. XXIV.[1] Isolation of Isodonal and Epinodosin from *Isodon japonicus* and Structure Elucidation of Sodoponin and Epinodosinol, Novel Diterpenoids of the Same Plant[2]

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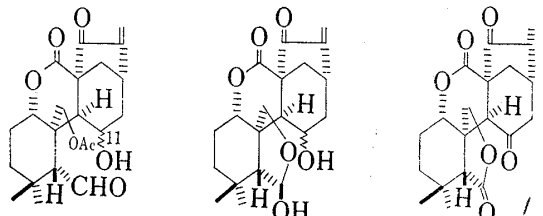
Four diterpenoids were isolated from the dried leaves and stems of *Isodon japonicus* Hara. Two of them were shown to be identical with the known isodonal (**2**) and epinodosin (**3**). Spectroscopic investigation and a chemical conversion into epinodosin dihydro-derivative (**10**) established the structure and absolute configuration of sodoponin as **11**. The structure and absolute configuration of epinodosinol were elucidated as **16**, on the basis of spectroscopic data and some chemical evidence. Finally, sodoponin (**11**) was converted into epinodosinol (**16**), which confirmed their structures unequivocally.

We had isolated enmein,[4] enmein-3-acetate,[4] isodocarpin,[5,6] nodosin,[5,7] isodotricin,[5,8] ponicidin,[5] and oridonin[9] from the dried leaves of *Iosodon japonicus* Hara ("Hikiokoshi," Labiatae), and elucidated their structures and absolute configurations except ponicidin.

Now, we isolated the other four diterpenoids in addition to the foregoing diterpenoids from the dried leaves and stems of the same plant.

One of them was obtained as needles, mp 230—233°. Its nuclear magnetic resonance (NMR) spectrum was very similar to that of trichodonin (**1**)[10] except 11-H signal, and hence, it was assumed to be isodonal, which was first isolated and shown to have structure **2** equivalent to 11-epitrichodonin by Kubota, *et al.*[11] Its comparison with the authentic sample of isodonal showed their identity.



1 : 11β-OH
2 : 11α-OH
3 : 11α-OH
5 : 11β-OH
4

The second diterpenoid was obtained as needles, mp 245—248°. It was converted into diacetate by acetylation and also into dihydro- and tetrahydro-derivatives by catalytic hydrogenation. On the basis of their spectroscopic investigation, it was assumed to be epinodosin (**3**) which had also been isolated and investigated by Kubota, *et al.*[10b] The assumption was proved to be correct by its comparison with the authentic sample of epinodosin.

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3) Location: *Uji, Kyoto-Fu, 611, Japan.*
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9) *a*) E. Fujita, T. Fujita, H. Katayama, and M. Shibuya, *Chem. Commun.*, **1967**, 252; *b*) E. Fujita, T. Fujita, H. Katayama, M. Shibuya, and T. Shingu, *J. Chem. Soc.* (*C*), **1970**, 1674; *c*) E. Fujita, T. Fujita, and H. Katayama, *ibid.*, **1970**, 1681.
10) *a*) E. Fujita, T. Fujita, and M. Shibuya, Symposium Papers of 10th Symposium Chemistry Natural Prod., Japan, 1965, p. 224; *b*) T. Kubota and I. Kubo, *Chem. Commun.*, **1968**, 763.

The structure and stereochemistry of epinodosin (**3**) had been determined by a conversion of isodonal dihydro-derivative into epinodosin dihydro-derivative by Kubota, *et al.*[10b] Now, we reconfirmed the structure and absolute configuration of epinodosin by the following fact. Epinodosin was converted into diketo-dilactone (**4**),[11] mp 192—195°, $[\alpha]_D^{27}$ −99°, *via* hydrogenation and subsequent Jones oxidation. This compound was proved to be identical with the product (**4**), mp 193—196°, $[\alpha]_D^{27}$ −95°, derived from nodosin, whose absolute configuration had been established as **5** by us,[7] by hydrogenation followed by Jones oxidation.

The other two minor compounds were found to be new diterpenoids and named sodoponin and epinodosinol.

The molecular formula $C_{22}H_{32}O_7$ was assigned for sodoponin, mp 229—231.5°, $[\alpha]_D^{28}$ +45.7°, on the basis of elemental analysis and molecular weight determination by high-resolution mass spectrometry. Its infrared (IR) absorptions at 3450, 3330, and 3250 cm^{-1} and at 1705 and 1267 cm^{-1} suggested the presence of hydroxy and acetoxy groups. In its NMR spectrum (100 MHz, in pentadeuteriopyridine) shown in Fig. 1, three doublets (Ha, Hc and He) were characterized as hydroxy protons by treatment with deuteroxide. The decoupling experiments clarified the couplings between Ha and Hl, Hc and Hh, and He and Hk. Thus, the presence of three secondary alcohol functions was suggested. The broad singlet (Hb) was also characterized as a hydroxy proton by deuteroxide, which was assigned to a tertiary alcohol function. The observation of a singlet (3H) at δ 1.98 ppm and a quartet (Hd) gave a suggestion for the presence of a secondary acetoxy group. An AB type signal (Hi and Hj) at δ 4.70 and 4.37 ppm was assignable to a methylene group between an ether-type oxygen and a tertiary carbon atom.

In addition to the foregoing functional groups, the presence of an exocyclic methylene (IR ν_{max} 1665 cm^{-1}, NMR δ 5.20 and 5.42 ppm) (Hf and Hg) and two tertiary methyl groups (NMR δ 1.10 and 1.14 ppm) was suggested.

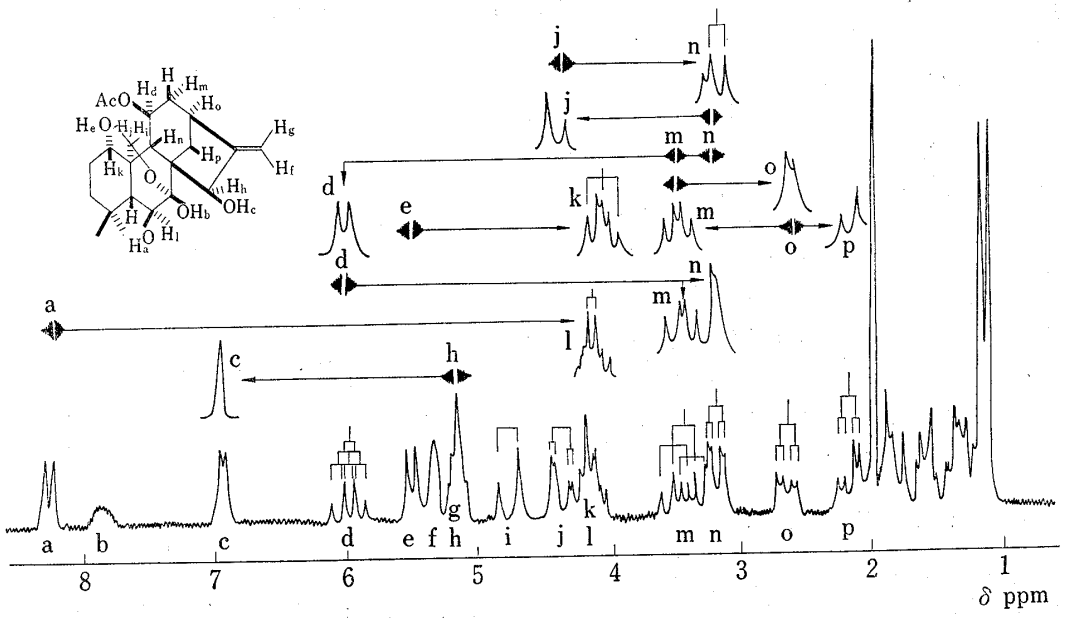
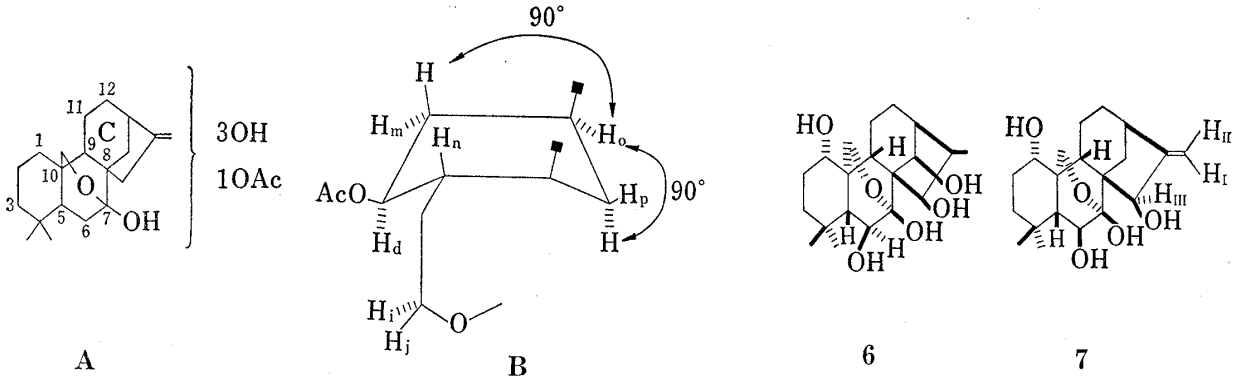


Fig. 1. The NMR Spectrum (100 MHz) of Sodoponin taken for Solution in d_5-pyridine with TMS as Internal Standard on a Varian HA-100 Spectrometer

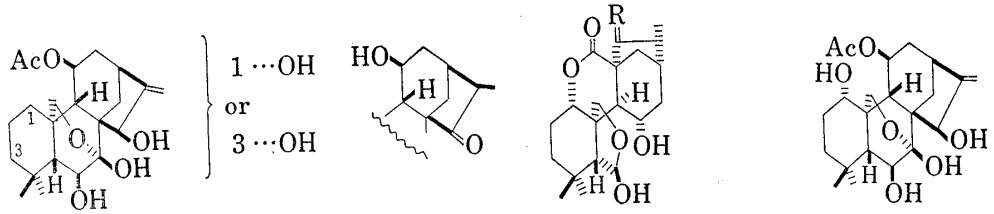
Sodoponin contains neither a five-membered ring hemiacetal, nor a lactone ring characteristic of enmein-type compounds, but it does have a tertiary hydroxy group. Consideration of all of the foregoing observations including the site of unsaturation led to an assumption of a kaurenetype structure (**A**) resembling that of trichokaurin[12] or oridonin.[9]

11) T. Kubota and I. Kubo, *Tetrahedron Letters*, **1967**, 3781.
12) E. Fujita, T. Fujita, M. Shibuya, and T. Shingu, *Tetrahedron*, **25**, 2517 (1969).



In the NMR spectrum (Fig. 1), the protons Hi and Hj can be easily assigned to C-20 methylene protons by analogy with the known kaurene-type diterpenoids. Then, the assignment of Hn to C-9 proton is possible, because of its long-range coupling to Hj.[13] Simultaneous irradiation on Hn (C-9 H) and Hm changed the Hd quartet to a doublet, which suggested the location of Hd, and therefore, of the acetoxy group in C-11, and also the location of Hm in C-12. Consideration of the coupling constants (J=9, 9, and 9 Hz) of the Hd quartet and the boat form of the C-ring suggested the quasi-equatorial conformation of the acetoxy group as shown in **B**. The couplings between Ho and Hm and between Ho and Hp were clarified by irradiations on Ho and Hm. As shown in Fig. 1, irradiation on Hm changed the Ho signal into a doublet (J=5 Hz), while irradiation on Ho changed the Hp signal into a doublet (J=12 Hz). These relationships can be reasonably explained by assignments of Hm to C-12 quasi-equatorial H, Ho to C-13 quasi-equatorial H, and Hp to C-14 quasi-equatorial H, because both of the dihedral angles between C-12 quasi-axial H and Ho and between Ho and C-14 quasi-axial H are about 90° and the couplings between these two couples would not be observed. Thus, the C-ring moiety is shown in **B**.

The triplet (Hl) at δ 4.21 ppm which changed to a doublet (J=5 Hz) by deuteroxide (D_2O) was assignable to C-6 quasi-equatorial H by analogy with the C-6 α H signal (d, J=5 Hz, by D_2O) at δ 4.20 ppm of the known compound **6**.[9b] Accordingly, OHa was assigned to C-6 quasi-axial OH. The multiplet (Hh) at δ 5.20 ppm was assigned to C-15-H by comparison with an apparent triplet ($J_{I,III}$=2.31 and $J_{II,III}$=2.85 Hz)[14] at δ 4.92 ppm assigned to H_{III} by the detailed NMR study of compound **7**.[14] The OHc is, therefore, located in C-15. The sextet (Hk) at δ 4.18 ppm which changed to a triplet (J=8 and 8 Hz) by D_2O was assigned to C-1 or C-3 axial H. Thus, structure (**8**) (or its antipode) was proposed for sodoponin.



8 9 10 : R=O 12 : R=α-OH, β-H 11

Now, sodoponin was treated with 15% methanolic hydrochloric acid to give a ketone **9**, mp 227—231°, by Garryfoline-Cuauchichicine rearrangement[15–17] accompanied by hy-

13) One of C-20 methylene protons *i.e.* the *pro*-R hydrogen is sterically located in 1,3-diaxial relationship to C-1 α hydroxy group. This proton should be subject to the paramagnetic shift, and hence, assignable to Hi rather than Hj. This assignment reasonably agrees with all the facts described in this paper.
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15) C. Djerassi, C.R. Smith, A.E. Lippman, S.K. Figdor, and H. Herran, *J. Am. Chem. Soc.*, **77**, 4801 (1955).
16) M.F. Barnes and J. MacMillan, *J. Chem. Soc.* (*C*), **1967**, 361.
17) E. Fujita, T. Fujita, M. Shibuya, and T. Shingu, *Tetrahedron*, **25**, 2517 (1969).

1H, AB-type, J=9, C-20 H_2), 3.11 (1H, s, C-5-H), 2.72 (1H, d, J=10, C-9-H), 1.05 (3H, d, J=6.5 Hz, C-16 Me), 0.98 (6H, s, C-4 Me_2); δ_{ppm}(d_5-pyridine+D_2O): 5.00 (1H, t, J=8.5 Hz, C-1-H), 4.80—4.30 (1H, m, C-11-H).

Recrystallization of 29 mg of the tetrahydro-derivative from MeOH gave 20 mg of pure sample, mp 242—246°, $[\alpha]_D^{27}$ −111° (c=0.01, pyridine). *Anal.* Calcd. for $C_{20}H_{30}O_6$: C, 65.55; H, 8.25; M 366.204. Found: C, 65.69; H, 8.49; M^+ *m/e* 366.206. IR ν_{max} cm^{-1}: 3340, 3200, 1710. NMR δ_{ppm}(d_5-pyridine): 5.72 (1H, s, C-6-H), 5.45 (1H, d, J=11, C-15-H), 4.82 (1H, t, J=9, C-1-H), 4.29 (1H, m, C-11-H), 4.39, 4.15 (each 1H, AB-type, J=9, C-20 H_2), 3.74 (1H, d, J=10, C-9-H), 3.10 (1H, s, C-5-H), 1.18 (3H, d, J=7 Hz, C-16-Me), 0.98 (6H, s, C-4 Me_2).

Diketo-dilactone 4——(a) To a solution of 35 mg of nodosin dihydro-derivative[7] in 3 ml of acetone (treated with $KMnO_4$) was slowly added Jones' reagent under stirring at 0° until the solution was kept yellowish. After stirring for 1 hr, MeOH was added. Neutralization with aq. Na_2CO_3, addition of acetone, filtration, and evaporation of the solvent from the filtrate *in vacuo* left a residue, which was extracted with AcOEt. A usual work-up gave 32 mg of a crystalline product, recrystallization (MeOH) of which yielded a pure sample of bisdehydro-derivative **4** as needles, mp 193—196°, $[\alpha]_D^{27}$ −95° (c=0.145, MeOH). *Anal.* Calcd. for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.90; H, 6.95. IR ν_{max} cm^{-1}: 1755, 1716. NMR δ_{ppm} ($CDCl_3$): 4.50 (1H, dd, J=6, 10, C-1-H), 4.22, 4.07 (each 1H, AB-type, J=10, C-20 H_2), 3.18 (1H, s, C-5-H), 2.61 (1H, s, C-9-H), 1.36 (3H, d, J=7 Hz, C-16-Me), 1.25, 1.04 (each 3H, s, C-4 Me_2). (b) Jones oxidation of epinodosin dihydro-derivative also yielded diketo-dilactone **4**, mp 192—195°, $[\alpha]_D^{27}$ −99° (c=0.088, MeOH). The identity of both compounds was confirmed by their IR, NMR, and mmp test.

Sodoponin (11)——The crude crystals, which precipitated from sodoponin-containing AcOEt solution obtained by column chromatography, were dissolved in pyridine, and, after filtration, MeOH was added. Concentration of the solution and recrystallization (MeOH) of the resulting precipitate gave pure sodoponin, mp 229—231.5°, $[\alpha]_D^{28}$ +45.7° (c=0.1, pyridine). *Anal.* Calcd. for $C_{22}H_{32}O_7$: C, 64.68; H, 7.90; M 408.215: M-CH_3CO_2H 348.193. Found: C, 64.81; H, 8.09; M^+ *m/e* 408.216; (M-CH_3CO_2H)$^+$ *m/e* 348.193. IR ν_{max} cm^{-1}: 3450, 3330, 3250 (OH), 1705 (OAc), 1665 (double bond), 1267 (OAc). NMR (100 MHz) δ_{ppm}(d_5-pyridine): 8.18 (1H, d, J=5, C-6-OHa), 7.91 (1H, br. s, C-7-OHb), 6.94 (1H, d, J=2, C-15-OHc), 5.99 (1H, q, J=9, 9, 9, C-11-Hd), 5.51 (1H, d, J=4, C-1-OHe), 5.42 (1H, s, C-17-Hf), 5.20 (2H, m, C-17-Hg) and C-15-Hh), 4.70 (1H, d, J=10, C-20-Hi), 4.37 (1H, dd, J=10, 2, C-20-Hj), 3.33 (1H, sextet, J=9, 9, 14, C-12-Hm), 3.08 (1H, dd, J=2, 9, C-9-Hn), 2.68 (1H, dd, J=5, 9, C-13-Ho), 2.18 (1H, dd, J=5, 12 Hz, C-14-Hp), 1.98 (3H, s, OAc), 1.14, 1.10 (each 3H, s, C-4 Me_2). Decoupling experiments: See the following Table.

Irradiated H	Ha	Hd	He
Result	Hl: d, J=5	Hm: dd, J=9, 14 Hn: d, J=2	Hk: t, J=8

Irradiated H	Hh	Hj	Hn
Result	Hc: s	Hn: d, J=9	Hj: d, J=10

Irradiated H(s)	Hm and Hn	Hm	Ho
Result	Hd: d, J=9	Ho: d, J=5	Hm: dd, J=9, 14 Hp: d, J=12 Hz

From the decoupling experiments shown above, the detailed patterns of Hk and Hl were clarified as follows δ_{ppm} (d_5-pyridine): 4.18 (1H, sextet, J=4, 8, 8, C-1-Hk), 4.21 (1H, t, J=5 Hz, C-6-Hl).

Acid Treatment of Sodoponin: Garryfoline-Cuauchichicine Rearrangement——To an ice-cooled and stirred solution of 34 mg of sodoponin in 20 ml of MeOH was dropwise added 15 ml of conc. HCl. The mixture was stirred at room temperature for 24 hr. After neutralization with aq. Na_2CO_3, MeOH was distilled off *in vacuo*. After extraction with $CHCl_3$, washing of the extract, and drying, the solvent was distilled off to leave 33 mg of crystalline residue, which was recrystallized from MeOH to give 20 mg of 15-oxo-16-dihydro-derivative **9**, mp 227—231°. *Anal.* Calcd. for $C_{20}H_{30}O_6$: M 366.204. Found: M^+ *m/e* 366.205. IR ν_{max} cm^{-1}: 3350, 1715, 1110. NMR δ_{ppm}(d_5-pyridine+D_2O): 4.68 (1H, q, J=10, 10, 10, C-11-H), 4.67, 4.45 (each 1H, AB-type, J=10, C-20 H_2), 4.22 (1H, d, J=7, C-6-H), 4.17 (1H, t, J=9 Hz, C-1-H); δ_{ppm}(d_5-pyridine): 6.8—5.5 (4H, br. s, 4×OH), 1.17 (3H, d, J=5 Hz, C-16-Me), 1.21, 1.13 (each 3H, s, C-4 Me_2).

Periodate Cleavage of 9: Formation of Epinodosin Dihydro-derivative (10)——To a solution of 20 mg of **9** in 5 ml of AcOH, a solution of 100 mg of $NaIO_4$ in 10 ml of H_2O was added. The mixture was stirred

at room temperature for 24 hr. After addition of saturated aq. NaCl, the mixture was extracted by AcOEt. The organic layer was washed with aq. Na_2CO_3 and saturated aq. NaCl and dried. The solvent was evaporated off to leave 13 mg of residue, which was crystallized from MeOH. Recrysrallization gave 9 mg of the pure epinodosin dihydro-derivative (**10**), mp 227—232°. IR ν_{max} cm^{-1}: 3380, 3320, 1760, 1720. The identity of this compound with the foregoing **10**, which was prepared by catalytic hydrogenation of epinodosin, was confirmed by mmp and IR comparison.

Epinodosinol (16)——The crude crystals were recrystallized (MeOH) to yield the pure epinodosinol as needles, mp 244—247°. (Recrystallization from a mixture of MeOH and pyridine gave crystals, mp 263—265°) $[\alpha]_D^{28}$ −87.5° (c=0.08, pyridine). *Anal.* Calcd. for $C_{20}H_{28}O_6$ M 364.189. Found: M^+ *m/e* 364.188. IR ν_{max} cm^{-1}: 3400, 3230 (OH), 1715 (δ-lactone), 1663 (double bond), 1060. NMR δ_{ppm}(d_5-pyridine): 5.71 (1H, s, C-6-H), 5.58 (1H, br. s, C-17-H), 5.54 (1H, br. s, C-15-H), 5.25 (1H, br. s, C-17-H), 4.87 (1H, t, J=8, C-1-H), 4.40 (1H, m, C-11-H), 4.53, 4.19 (each 1H, AB-type, J=9, C-20 H_2), 3.57 (1H, d, J=10 Hz, C-9-H), 3.17 (1H, s, C-5-H), 1.00 (6H, s, C-4 Me_2).

Catalytic Hydrogenation of Epinodosinol——To a solution of 7 mg of epinodosinol in 4 ml of MeOH was added 8 mg of PtO_2 and the mixture was stirred for 4 hr in the atmosphere of hydrogen. After filtration, the solvent was evaporated off to leave 7 mg of residue, which was chromatographed (SiO_2, CH_2Cl_2: acetone= 9:1). Earlier eluant which was obtained as 3.5 mg of crystals was purified by recrystallization from MeOH to yield 2 mg of the pure compound, mp 228—232°. *Anal.* Calcd. for $C_{20}H_{28}O_6$: M 364.189. Found: M^+ *m/e* 364.187. IR ν_{max} cm^{-1}: 3380, 3320, 1760, 1720. Comparison of its IR spectrum with that of epinodosin dihydro-derivative (**10**) and mmp confirmed both compounds to be identical. Later eluant gave 5 mg of another crystalline product, recrystallization (MeOH) of which yielded 3 mg of the pure compound, mp 243—245°, $[\alpha]_D^{27}$ −99° (c=0.016, pyridine). *Anal.* Calcd. for $C_{20}H_{30}O_6$: M 366.204. Found: M^+ *m/e* 366.205. IR ν_{max} cm^{-1}: 3340, 3200, 1710. The IR spectrum of this compound was superimposable with that of the foregoing epinodosin tetrahydro-derivative (**12**), and mmp test confirmed the identity of both compounds.

Sodium Borohydride Reduction of 15 to Epinodosin Tetrahydro-derivative (12)——To a suspension of 65 mg of $NaBH_4$ in 2.5 ml of isopropyl alcohol was dropwise added a solution of 35 mg of the ketone **15** in 7.5 ml of isopropyl alcohol under stirring, and the mixture was stirred for 2 days. The TLC test showed only one spot. After neutralization with 3.6% HCl, evaporation of the solvent *in vacuo* left a residue, which was suspended in H_2O and extracted with AcOEt. The extract was treated as usual to give 20 mg of a crude crystalline product, which was recrystallized (MeOH) to yield 6 mg of the pure compound. This compound was proved to be identical with the foregoing epinodosin tetrahydro-derivative (**12**) (TLC, IR, mmp).

Conversion of Sodoponin (11) into Epinodosinol (16)——To a solution of 30 mg of sodoponin (**11**) in 2 ml of AcOH was added an aq. suspension of $NaIO_4$ (150 mg in 4 ml) and the mixture was stirred at room temperature for 3 days. Evaporation of the solvent left a residue, which, after addition of H_2O, was extracted with AcOEt. The organic layer was washed with aq. NaCl solution and dried. Evaporation of the solvent left 29 mg of residue, whose TLC showed a few spots. Column-chromatography (SiO_2, CH_2Cl_2: acetone= 9: 1) isolated 15 mg of a crude crystalline substance, mp 230—241°. IR ν_{max} cm^{-1}: 3380, 3320, 1718, 1693, 1280. Six mg of the crude crystals was dissolved in 1 ml of MeOH and an aq. K_2CO_3 (12 mg in 0.5 ml) was added to this solution. After 10 min, 4 ml of a saturated aq. NaCl was added, and MeOH was removed by evaporation *in vacuo*. Extraction with $CHCl_3$, washing of the extract with aq. NaCl solution, drying of the $CHCl_3$ solution, and evaporation of the solvent left 4 mg of residue, which was crystallized (MeOH) to yield 2 mg of needles, mp 242—246°, $[\alpha]_D^{27}$ −80° (c=0.02, pyridine). *Anal.* Calcd. for $C_{20}H_{28}O_6$: M 364.189. Found: M^+ *m/e* 364.190. IR ν_{max} cm^{-1}: 3400, 3230 (OH), 1715 (δ-lactone), 1663 (double bond), 1060. The identity of this compound with epinodosinol (**16**) was established by a comparison of IR spectra of both compounds and mmp.

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