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# Protosappanin A, a Novel Biphenyl Compound from Sappan Lignum

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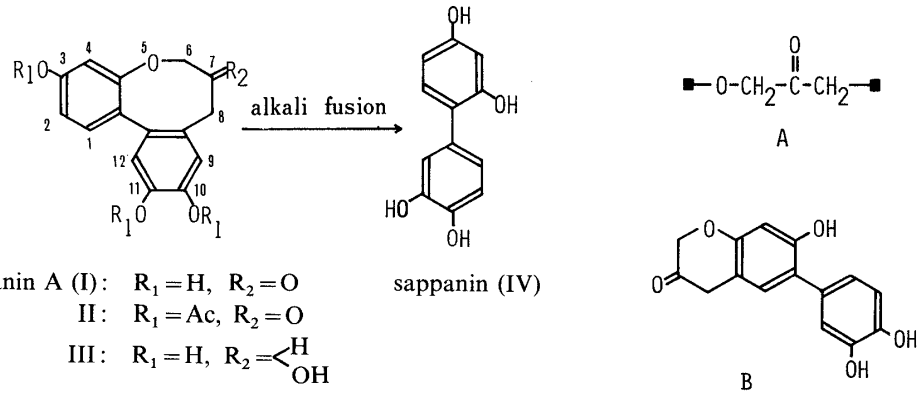
Protosappanin A (I), $C_{15}H_{12}O_5$, mp 250—251 °C, was isolated from the heart-wood of *Caesalpinia sappan* L. (Sappan Lignum). It yielded a triacetate (II) on acetylation, and an alcohol (III) on reduction with sodium borohydride. On alkali fusion, I afforded sappanin (IV) along with small amounts of catechol and resorcinol. On the basis of chemical and spectroscopic evidence, the structure of I was established as 3,10,11-trihydroxy-7,8-dihydro-6*H*-dibenz[*b*,*d*]oxocin-7-one. The result of an X-ray crystal structure analysis supported this conclusion. Protosappanin A (I) seems to be the precursor of sappanin, 2,3′,4,4′-tetrahydroxybiphenyl, which has been known as an artificial component of Sappan Lignum since 1872, and may be a metabolite of sappanchalcone. A possible biogenetic relationship of sappanchalcone, brazilin, and protosappanin A is discussed. Protosappanin A (I) has a weak sedative effect in mice.

**Keywords**——Leguminosae; *Caesalpinia sappan*; Sappan Lignum; biphenyl; oxocin; protosappanin A; X-ray crystallographic analysis; sappanin; sedative effect

Sappan Lignum is the dried heart-wood of *Caesalpinia sappan* L. (Leguminosae). This heart-wood has been used as a red dyestuff for a long time. Extracts of Sappan Lignum reportedly have some pharmacological activities[1] such as a depressing effect on the central nervous system. We demonstrated that the methanol extract of Sappan Lignum has a sleeping-time-elongating effect in mice and we began an investigation of the chemical component(s) responsible for the sedative effect. We previously reported on a chalcone methyl ether, sappanchalcone,[2] which seems to be the biosynthetic precursor of brazilin. This paper deals with the isolation and structure elucidation of a novel biphenyl compound, designated as protosappanin A (I), which seems to be a metabolite of sappanchalcone and the precursor of sappanin.

Protosappanin A, $C_{15}H_{12}O_5$, mp 250—251 °C, optically inactive, showed absorptions ascribable to hydroxyls (3500—3100 $cm^{-1}$), a carbonyl (1695 $cm^{-1}$), and aromatic groups (1605, 1495 $cm^{-1}$) in its infrared (IR) spectrum, and gave a triacetate (II), $C_{21}H_{18}O_8$, mp 183—184 °C, on acetylation. No hydroxyl absorption was observed in the IR spectrum of II. The carbon-13 nuclear magnetic resonance ($^{13}C$-NMR) spectrum of I showed fifteen signals comprising two benzene rings (seven singlets and five doublets), two methylenes at $\delta$ 49.4 ppm

and $\delta$ 78.9 ppm, and a ketonic carbon at $\delta$ 206.0 ppm. Taking into consideration the presence of three hydroxyls, a carbonyl and a methylene resonating at $\delta$ 78.9 ppm, one of the five oxygens of I was concluded to form an ether linkage between the methylene and one of the two benzene rings. In the proton nuclear magnetic resonance ($^1$H-NMR) spectrum, II showed two overlapped singlets at $\delta$ 7.18 ppm [1,2,4,5-tetrasubstituted benzene] and an ABX pattern at $\delta$ 7.02 ppm (d), $\delta$ 7.05 ppm (dd), and $\delta$ 7.38 ppm (d) [1,2,4-trisubstituted benzene], two two-proton singlets at $\delta$ 3.59 ppm and $\delta$ 4.56 ppm, and three three-proton singlets ascribable to three acetyl groups.



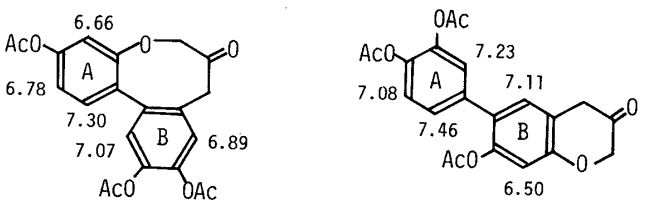

protosappanin A (I): $R_1$=H, $R_2$=O
II: $R_1$=Ac, $R_2$=O
III: $R_1$=H, $R_2$=<H/OH

sappanin (IV)

Chart 1

Sodium borohydride reduction of I provided a dihydro derivative (III), mp 292—294 °C, whose IR spectrum showed no carbonyl absorption. In the $^1$H-NMR spectrum of III, two two-proton multiplets at $\delta$ 3.12 ppm and $\delta$ 4.20 ppm and a one-proton multiplet at $\delta$ 4.85 ppm were observed. The two-proton multiplet at high magnetic field ($\delta$ 3.12 ppm) changed into a doublet on measurement at higher temperature (90 °C). It follows that the aliphatic part of I has the partial structure A (Chart 1).

Protosappanin A (I) is unstable even to weak alkali such as 1% aqueous sodium bicarbonate. On the other hand, Schreder[3] isolated sappanin (IV) in addition to catechol and resorcinol upon alkaline treatment of the extract of Sappan Lignum. By using his procedure, we obtained a poor yield of IV, $C_{12}H_{10}O_4$, mp 202—203 °C, positive colorations with ferric chloride (pink) and sodium hypochlorite (dark green). The spot of sappanin (IV) on the thin layer chromatogram (TLC) (silica gel) took a characteristic green color on exposure to air. Alkali fusion of protosappanin A (I) was also tried, and yielded sappanin (IV) accompanied by small amounts of catechol, resorcinol, and an unknown substance. Sappanin (IV) has been known as a component of Sappan Lignum since 1872. Its unique structure suggests that IV is probably an artificial product, and in fact Schreder obtained IV after alkali fusion of the extract. Although the origin of IV in the heart-wood has been unclear for a long time, at least one of the precursors of IV was clarified here to be protosappanin A (I).




triacetate of I triacetate of B

Chart 2. Calculated $^1$H-NMR Chemical Shifts

On the basis of all the above findings protosappanin A (I) must have the same biphenyl structure as sappanin (IV) and the partial structure A (Chart 1). A Dreiding model showed two possible structures I and B (Chart 1) for protosappanin A. In order to identify the correct structure of protosappanin A, the $^1$H-NMR spectrum of the triacetate (II) was analyzed in detail. Chemical shifts of aromatic ring protons were calculated on the basis of the additivity rule of substituent effects,[4] and are given in Chart 2 for the acetates of I and B. The observed chemical shifts of II are in better agreement with the calculated ones for the triacetate of I than with those for the triacetate of B. Two overlapped singlets were observed at $\delta$ 7.18 ppm: in the triacetate of B two singlets ascribable to protons on the B-ring are expected to show a large difference ($\delta$ 0.61 ppm) between their chemical shifts. The three protons on the A-ring were observed as an ABX pattern at $\delta$ 7.02 ppm (d, $J$=2.2 Hz), $\delta$ 7.05 ppm (dd, $J$=2.2 and 8.8 Hz) and $\delta$ 7.38 ppm (d, $J$=8.8 Hz): in the triacetate of B one of the three protons resonating at the highest magnetic field ($\delta$ 7.08 ppm) should be observed as a doublet with a coupling constant of about 8 Hz, and another one at the lowest magnetic field ($\delta$ 7.46 ppm), as a double-doublet with two coupling constants of about 2 and 8 Hz. Consequently the chemical structure of protosappanin A (I) was established as 3,10,11-trihydroxy-7,8-dihydro-6*H*-dibenz[*b*,*d*]-oxocin-7-one (Chart 1).

In order to confirm the structure of protosappanin A (I), an X-ray structure analysis was undertaken. Crystals of I were grown in a methanol–water solution as colorless prisms. The molecular structure of protosappanin A (I) was determined to be as shown in Fig. 1, and this result is in agreement with the above conclusion based on the chemical and physicochemical evidence. The final atomic parameters are presented in Table I together with standard deviations. The bond lengths and angles, and the numberings are shown in Fig. 2. These values are not significantly different from the expected ones. The torsion angles C(12)–C(12a)–C(12b)–C(1) and C(8a)–C(12a)–C(12b)–C(4a) are $-58.9°$ and $-59.7°$, respectively,

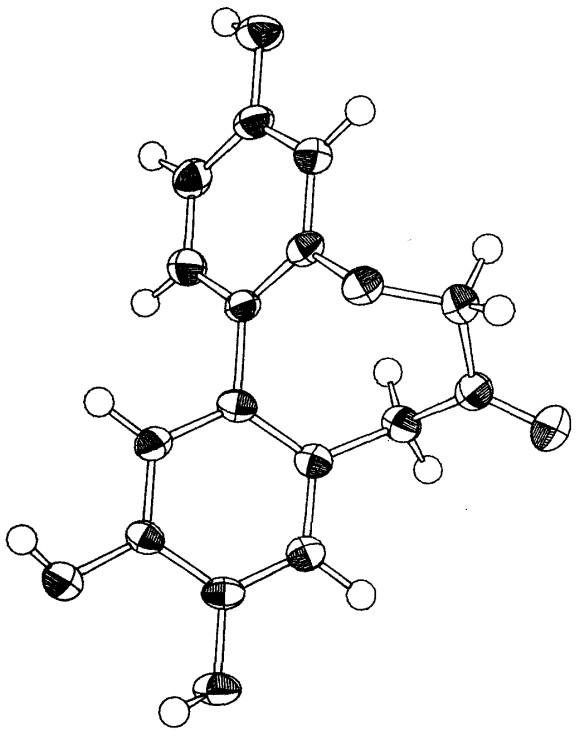


Fig. 1. The Molecular Structure of Protosappanin A

Thermal ellipsoids of non-hydrogen atoms are drawn at the 50% probability level.

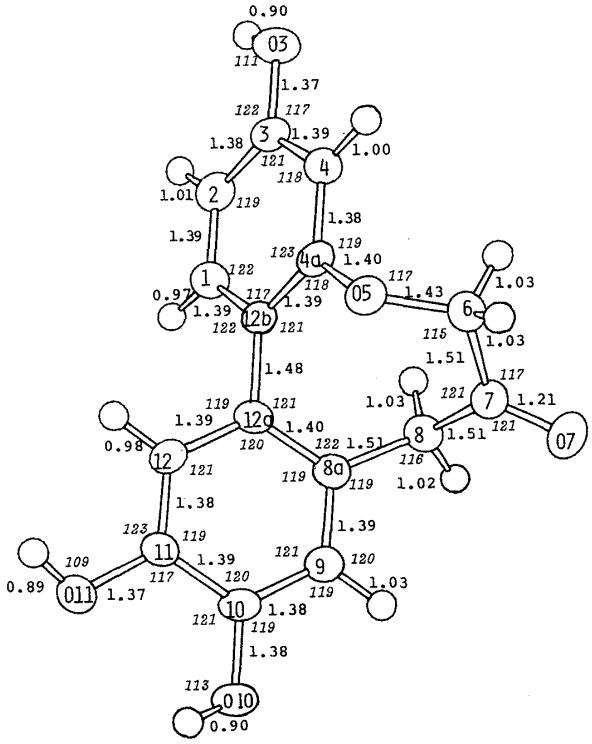



Fig. 2. Structure and Numbering Scheme of Protosappanin A

Bond lengths (Å) and angles (°) are shown.

TABLE I. Final Atomic Parameters ($\times 10^4$) and Equivalent Thermal Parameters, with Estimated Standard Deviations in Parentheses

| Atom | $x$ | $y$ | $z$ | $B_{eq}$ or $B$ |
|---|---|---|---|---|
| C(1) | 2392 (2) | 3296 (1) | 65 (4) | 2.8 |
| C(2) | 2152 (2) | 2660 (1) | 235 (4) | 2.9 |
| C(3) | 2744 (2) | 2271 (1) | 1107 (3) | 2.7 |
| C(4) | 3575 (2) | 2509 (1) | 1808 (3) | 2.5 |
| C(4a) | 3791 (2) | 3142 (1) | 1604 (3) | 2.2 |
| C(6) | 4708 (2) | 3500 (1) | 3825 (3) | 2.7 |
| C(7) | 4244 (2) | 4112 (1) | 4342 (3) | 2.5 |
| C(8) | 3381 (2) | 4384 (1) | 3501 (3) | 2.6 |
| C(8a) | 3561 (2) | 4625 (1) | 1869 (3) | 2.2 |
| C(9) | 3783 (2) | 5261 (1) | 1646 (3) | 2.3 |
| C(10) | 3928 (2) | 5503 (1) | 174 (3) | 2.1 |
| C(11) | 3857 (2) | 5108 (1) | −1124 (3) | 2.2 |
| C(12) | 3617 (2) | 4479 (1) | −918 (3) | 2.3 |
| C(12a) | 3474 (2) | 4230 (1) | 567 (3) | 2.2 |
| C(12b) | 3216 (2) | 3550 (1) | 745 (3) | 2.2 |
| O(3) | 2567 (2) | 1638 (1) | 1282 (2) | 3.7 |
| O(5) | 4662 (1) | 3377 (1) | 2192 (2) | 2.4 |
| O(7) | 4552 (2) | 4354 (1) | 5523 (2) | 3.7 |
| O(10) | 4102 (1) | 6144 (1) | 15 (2) | 2.9 |
| O(11) | 4008 (2) | 5376 (1) | −2551 (2) | 3.0 |
| H(C1) | 1975 (25) | 3567 (17) | −562 (43) | 0.8 (0.8) |
| H(C2) | 1550 (21) | 2499 (17) | −300 (37) | 0.5 (0.7) |
| H(C4) | 3985 (24) | 2212 (15) | 2436 (41) | 0.8 (0.7) |
| H(C6-1) | 4403 (22) | 3134 (14) | 4457 (37) | 0.4 (0.7) |
| H(C6-2) | 5430 (24) | 3522 (16) | 4089 (40) | 1.2 (0.8) |
| H(C8-1) | 3076 (23) | 4744 (15) | 4149 (39) | 1.4 (0.8) |
| H(C8-2) | 2861 (20) | 4042 (13) | 3498 (34) | 0.5 (0.6) |
| H(C9) | 3747 (21) | 5556 (13) | 2593 (36) | 0.5 (0.6) |
| H(C12) | 3501 (24) | 4190 (15) | −1797 (39) | 1.1 (0.8) |
| H(O3) | 1983 (32) | 1536 (22) | 889 (54) | 4.2 (1.2) |
| H(O10) | 4457 (30) | 6237 (20) | −830 (51) | 4.0 (1.1) |
| H(O11) | 4138 (30) | 5273 (20) | −3240 (49) | 2.9 (1.1) |

and the dihedral angle between the least-squares planes formed by the two aromatic rings of the biphenyl moiety is 59.5°. The intermolecular non-bonding distances of 2.746, 2.758, and 2.819 Å found between O(10) and O(5); O(10) and O(3); and O(11) and O(7), and those of 1.658, 1.871, and 1.925 Å found between H(O10) and O(5); O(10) and H(O3); and H(O11) and O(7), may represent hydrogen bonds of OH---O type. No intramolecular hydrogen bonding was observed. The molecules are packed together mainly through the hydrogen bonding between the molecules of I in the crystals.

Recently, a monohydroxybrazilin[5] and benzyldihydrobenzofuran derivatives[6] have been isolated from Sappan Lignum. All of these compounds have sixteen skeletal carbons, while protosappanin A (I) contains fifteen carbons. Though the biosynthetic route to brazilin has already been discussed by Dewick,[7] the co-occurrence of sappanchalcone, the monohydroxybrazilin, the benzyldihydrobenzofurans and protosappanin A in the heart-wood suggests some biogenetic relation. We propose the following biosynthetic scheme (Chart 3). Starting from sappanchalcone, a 1,2-diol (D)[5] is formed *via* a homoisoflavone (C). Brazilin is a metabolite of D formed through dehydration to brazilein followed by hydrogenation of brazilein. On the other hand, an acyloin (E) is derived from the 1,2-diol (D) by retro-aldol type ring opening. Bond splitting between the carbonyl and the alcoholic hydroxy of E affords

a keto-aldehyde (F), which is the precursor of both the benzyldihydrobenzofurans[6] and protosappanin A. Protosappanin A is biosynthesized from F through oxidative coupling between the two phenol rings to an oxocin (G), followed by retro-Claisen type elimination of the formyl group from G.

Protosappanin A extended the sleeping time of mice induced by hexobarbital (80 mg/kg) at the dose of 42 mg/kg (*i.p.*), but its activity seems to be relatively minor in relation to the sleeping-time-elongating effect of the methanol extract of Sappan Lignum.

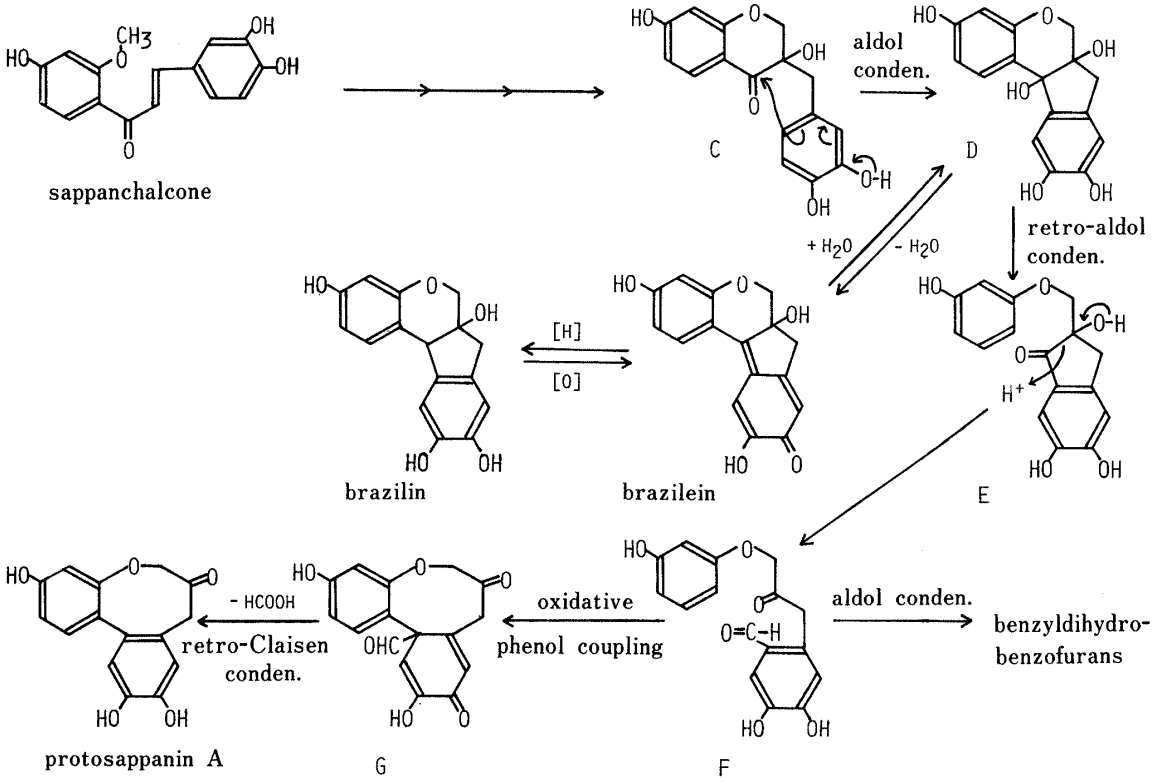



Chart 3

## Experimental

All melting points were taken on a Shimadzu micro melting point determination apparatus and are uncorrected. NMR spectra were recorded a JEOL JNM FX-100 spectrometer with tetramethylsilane as an internal standard. Chemical shifts are given on the $\delta$ scale (ppm) and coupling constants ($J$ values) are expressed in Hz. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, dd=double doublet, m=multiplet. Mass spectrum (MS) were recorded with a JEOL JMD-D 300 machine. IR and ultraviolet (UV) spectra were obtained with a Shimadzu IR-400 spectrometer and a Shimadzu UV-250 spectrometer, respectively. Thin-layer chromatography (TLC) was performed on Kieselgel 60 $F_{254}$ precoated plates (Merck) unless otherwise stated, and detection was carried out by spraying 10% $H_2SO_4$ followed by heating and by UV irradiation (254 nm).

**Extraction and Isolation of Protosappanin A (I)**——Sappan Lignum (500 g) was obtained in Tokyo market in 1984 and was extracted six times with MeOH for 2 h each under reflux. The total MeOH solution was concentrated under reduced pressure, affording the extract (127 g). A part of the extract (39 g) was repeatedly chromatographed on silica gel with $CHCl_3$–MeOH–$H_2O$ (800 : 150 : 8) (solvent A) as the developing solvent. Some fractions eluted with solvent A gave a crude crystalline substance. After being washed with MeOH, it was recrystallized from MeOH–$H_2O$ (1 : 1) to give protosappanin A (I) as colorless needles (94.6 mg). I, mp 250—251 °C. *Anal.* Calcd for $C_{15}H_{12}O_5$: C, 66.17; H, 4.44. Found: C, 65.84; H, 4.38. $^{13}$C-NMR (acetone-$d_6$) $\delta$: 49.4 (–$CH_2$–), 78.9 (O–$CH_2$–), 125.1, 127.2, 131.9, 145.4, 145.7, 159.2, 159.8 (each s), 109.2, 113.5, 117.5, 117.7, 131.1 (each d), 206.0 (>C=O). $^1$H-NMR (acetone-$d_6$) $\delta$: 3.40 (2H, s), 4.47 (2H, s), 6.6—6.85 (4H, m), 7.24 (1H, d, $J$=9). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3500—3100, 1695, 1605, 1495, 810. MS $m/z$: 272 ($M^+$), 244, 229. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 260 (4.00), 284 (3.84).

**Triacetate (II)**——A mixture of protosappanin A (15 mg), $Ac_2O$ (1 ml) and pyridine (2 ml) was allowed to stand overnight at room temperature. After work-up in the usual manner, the product was passed through a silica gel column (3 g) (eluent, benzene–EtOAc (9 : 1)). II, colorless crystalline powder (9 mg), mp 183—184 °C. MS $m/z$: 398

($M^+$), 356, 314, 272, 244, 43. High MS *m/z*: Calcd for $C_{21}H_{18}O_8$ ($M^+$) 398.100. Found: 398.089. UV $\lambda_{max}^{MeOH}$ nm ($\log\varepsilon$): 248 (4.01), 281 (3.52). $^1$H-NMR ($CDCl_3$) $\delta$: 2.30, 2.31, 2.33 (3H, each s, $CH_3COO$–), for others see the text. $^{13}$C-NMR ($CDCl_3$) $\delta$: 20.6 (×2), 21.1 (each q, $\underline{C}H_3COO$–), 49.0 (–$CH_2$–), 77.6 (O–$CH_2$–), 114.8, 118.7, 124.2, 124.6, 129.9 (each d), 130.6 (×2), 136.4, 141.4 (×2), 151.9, 157.1 (each s), 167.9, 168.0, 168.9 (each s, $CH_3\underline{C}OO$–), 203.4 (>C=O).

**Reduction of Protosapanin A (I)**——Sodium borohydride (20 mg) was added to a solution of I (23.6 mg) in MeOH (1 ml) with stirring. The reaction mixture was allowed to stand overnight at room temperature. Several drops of AcOH and $H_2O$ (10 ml) were added to the solution, then the MeOH was evaporated off under reduced pressure. The residual water solution was extracted with EtOAc, and the EtOAc layer was washed with water and dried over anhydrous $Na_2SO_4$. After concentration of the EtOAc solution, the residue was applied to a column of silica gel (4 g) and the column was eluted with solvent A, affording an alcohol (III). III, colorless needles (MeOH–$H_2O$), mp 292—294 °C. MS *m/z*: 274 ($M^+$), 229, 213. High MS *m/z*: Calcd for $C_{15}H_{14}O_5$ ($M^+$) 274.084. Found: 274.081. $^1$H-NMR ($C_5D_5N$): 3.12 (2H, m, 8-$H_2$), 4.20 (2H, m, 6-$H_2$), 4.85 (1H, m, 7-H). $^1$H-NMR ($C_5D_5N$, 90 °C) $\delta$: 2.99 (2H, d, $J$=5.6 Hz, 8-$H_2$).

**Alkali Fusion of the MeOH Extract of Sappan Lignum**——The MeOH extract (2 g) was added to a mixture of KOH (8 g) and $H_2O$ (5 ml) in a metal crucible and the mixture was heated at about 210 °C for 30 min by direct application of a gas flame. Using fresh portions of the MeOH extract, this alkali fusion was repeated seven times. The total reaction mixture was diluted with $H_2O$ and extracted with ether after being acidified with 6 N HCl. The ether layer was washed with $H_2O$, dried over anhydrous $Na_2SO_4$ and concentrated. The residue was chromatographed over silica gel (30 g). Elution with solvent A gave sappanin (IV), 5.8 mg. IV, colorless needles (from $H_2O$), mp 202—203 °C (lit.[10] mp 201—202 °C). High MS *m/z*: Calcd for $C_{12}H_{10}O_4$ ($M^+$) 218.058. Found: 218.059. Color reactions: pink with 10% $FeCl_3$ and dark green with sodium hypochlorite.

**Alkali Fusion of Protosappanin A (I)**——I (59.2 mg) was added to a mixture of KOH (200 mg) and $H_2O$ (0.13 ml), and alkali fusion was carried out for 5 min in the same way as in the case of the MeOH extract. The reaction mixture (16 mg) was chromatographed on TLC using solvent A, and four spots were detected. Three of them were identical with authentic samples. *Rf* values: 0.63 (catechol); 0.52 (resorcinol); 0.30 (unknown); 0.25 (sappanin).

**X-Ray Crystallographic Analysis of Protosappanin A (I)**——i) Crystal Data: *Pbca* (orthorhombic); $a$=13.836 (2), $b$=21.081 (6), $c$=8.593 (2) Å; $V$=2506.4 (10) Å$^3$; $Z$=8; $D_c$=1.44 g/cm$^3$; $F(000)$=1136. The diffraction intensities were collected from a crystal of dimensions 0.8×0.2×0.2 mm on a Rigaku AFC-5 FOS four-circle diffractometer using Mo$K_\alpha$ radiation monochromated by means of a graphite plate. A total of 1804 reflections were measured in a $2\theta$ range of 2—50° as being above the $2\sigma(F)$ level. These were used in the solution and refinement of the structure.

ii) Determination of the Structure: The structure was solved by the direct method using MULTAN[8] and refined by the block-matrix least-squares method. In the final refinement, anisotropic thermal parameters were used for all non-hydrogen atoms, and isotropic thermal parameters for hydrogen atoms. The final $R$ factor with hydrogen atoms was 0.047. The data were not corrected for the effects of absorption. The final atomic parameters are shown in Table I, and bond lengths and angles in Fig. 2.[9]

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