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Studies on the Constituents of *Sambucus chinensis* Lindl.

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From the leaves of *Sambucus chinensis* Lindl. were isolated α-amyrin palmitate, ursolic acid and β-sitosterol which was confirmed to contain small amounts of stigmasterol and campesterol by gas–liquid chromatography. α-Amyrin palmitate was the first instance which was obtained as crystals from the natural products. A relatively large amount of potassium nitrate was separated from the water soluble fraction.

Since the authors[2] reported in the previous paper that ursolic acid and β-sitosterol were obtained from the leaves of *Sambucus sieboldiana* Blume, triterpenoids in some *Sambucus* species have been studied. From the non–saponifiable fraction of the above leaves Tsukamoto, *et al.*[3] obtained α-amyrin and β-sitosterol which was elucidated to contain stigmasterol and campesterol by gas–liquid chromatography (GLC). Lawrie, *et al.*[4] isolated α-amyrin, α–amyrone, betulin, oleanolic acid, β-sitosterol and ceryl alcohol from the non–saponifiable fraction of the bark of *S. nigra* L., while Huneck and Snatzke[5] obtained ursolic acid and *n*-heptacosane from the bark of *S. nigra* L., and betulic acid and *n*-nonacosane from the bark of *S. racemosa* L. respectively, together with α-amyrin, betulin, β–sitosterol and ceryl alcohol.

The leaves of *S. chinensis* Lindl. (Japanese name: Sokuzu) is one of the Oriental crude drugs as that of *S. sieboldiana* Blume but the chemical constituents have not been studied. In present paper the constituents in the leaves of *S. chinensis* Lindl. were studied in the pharmacological and chemotaxonomic interest.

The methanol extract of the leaves was extracted with hexane (Fr. I), subsequently with ether (Fr. II), then with hot water (Fr. III) and filtered to remove insoluble residue (Fr. IV). Hexane extract (Fr. I) was chromatographed on alumina and eluted with (a) hexane, (b) hexane–ether (1:1) and (c) ether. The fraction (b) was repeatedly chromatographed on alumina and silica gel to yield waxy colorless crystals, which were recrystallized from ethanol–ether to give prisms or needles, $C_{46}H_{80}O_2$, mp 73—74°, $[\alpha]_D^{23}$ +52.5° (c=1.0, benzene).

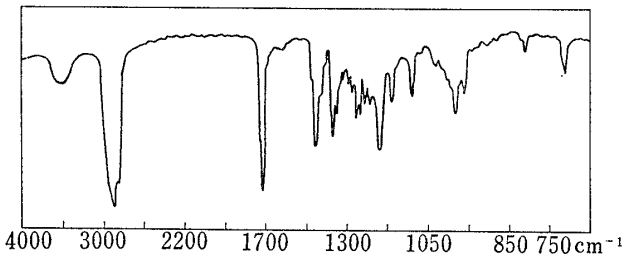

Fig. 1. IR Spectrum of α-Amyrin Palmitate (KBr)

As the results that the crystal showed positive Liebermann–Burchard reaction and the infrared (IR) spectrum (KBr) had the absorption bands at 1730 and 1175 cm^{-1}, and the band progression similar to that of long–chain fatty acids[6] in the region 1350—1180 cm^{-1} as shown in Fig. 1, it was suggested to be a fatty

1) Location: *Ebara 2–chome, Shinagawa–ku, Tokyo.*
2) T. Inoue, M. Moriguchi and R. Aoyama, *Hoshi Yakka Daigaku Kiyo*, **11**, 17 (1962).
3) T. Tsukamoto, A. Yagi, K. Mihashi, T. Kawasaki and I. Nishioka, Abstracts of Papers, the Annual Meeting of Pharmacognosical Society of Japan, Tokyo, May, 1965, p. 22.
4) W. Lawrie, J. McLean and A.C. Paton, *Phytochemistry*, **3**, 267 (1964).
5) S. Huneck and G. Snatzke, *Chem. Ber.*, **98**, 120 (1965); *Phytochemistry*, **4**, 777 (1965).
6) R.G. Sinclair, A.F. McKay and N. Jones, *J. Am. Chem. Soc.*, **74**, 2570, 2575 (1952).

acid ester of triterpene. On alkaline hydrolysis of the crystals the neutral portion gave α-amyrin, mp 180°, as colorless needles and the acidic portion afforded palmitic acid, mp 60—61°, as colorless leaflets. In addition methyl ester of the acid was confirmed to be identical with the authentic methyl palmitate by GLC. Consequently original crystals were elucidated to be α-amyrin palmitate. A sample solution of α-amyrin palmitate was prepared from α-amyrin and palmitoyl chloride to compare with our α-amyrin palmitate by thin-layer chromatography (TLC) and both samples gave perfectly the same spots in *Rf* values and color reaction on chromatoplate. α-Amyrin palmitate is soluble in hexane, ether and benzene, slightly soluble in ethanol and insoluble in water.

Recrystallization of fraction (c) yielded phytosterol as colorless plates, mp 138°. The sterol and its acetate showed no depression in mixed melting point with β-sitosterol and its acetate respectively but GLC of sterol proved that it was β-sitosterol containing small amounts of stigmasterol and campesterol similarly to a sterol of *S. sieboldiana*.

A compound from ether extract (Fr. II) and water insoluble residue (Fr. IV) was obtained as colorless needles, mp 278—279°, giving positive Liebermann-Burchard reaction, which were identified with ursolic acid.

The water soluble fraction (Fr. III), which was treated with lead acetate and basic lead acetate, was subsequently concentrated *in vacuo* to yield a large amount of crystals. The crystals were recrystallized from water to give colorless prisms, mp 330°, which were identified with potassium nitrate.

Regarding long-chain fatty acid esters of triterpene the following compounds were recorded but these are waxy substances and perhaps could not be obtained as crystal. β-Amyrin palmitate, also known as balanophorin, was isolated from the resin of *Balanophora elongata*,[7] the leaves of *Erythroxylon coca* var. *novogranatense*[8] and the latex of *Ficus variegata*.[9] β-Amyrin stearate was found to be present in the latex of *F. alba*,[10] β-amyrin myristate in a Coca leaves.[11] Many workers have studied on the components of bird-lime, in which triterpenes were known to be existent as fatty acid esters.[12] Although they obtained some kinds of triterpenes such as α- and β-amyrin, betulin and lupeol from the non-saponifiable fraction and mainly palmitic acid from the acid fraction on hydrolysis of bird-lime, no attempt to obtain original esters seems to have been done because of the difficulty in purification. α-Amyrin palmitate was the first instance which was isolated as crystals from the natural products.

It has been known that the leaves of *S. chinensis* Lindl. is applied as a medicated baths or a external decoctions for analgesic of rheumatism and tumor, and the probable components concerned with the above pharmacological action could not be found but the occurrence of relatively large amount of potassium nitrate suggested that the decoctions of this leaves have the diuretic action similar to that of *S. sieboldiana* Blume.

Experimental[13]

Extraction of *S. chinensis* Lindl.——The dried leaves (1.0 kg) which were collected at Nishitama district of Tokyo in June of 1967 were extracted four times for four hours with hot MeOH and the extract was concentrated *in vacuo* to syrup. The MeOH extract was extracted with hexane (Fr. I), subsequently with ether (Fr. II), then treated with hot water and separated to water soluble portion (Fr. III) and water insoluble residue (Fr. IV).

7) M. Simon, *Monatsch. Chem.*, **32**, 89 (1911); A.J. Ultée, *Bull. Jardin Botan. Buitenzorg* [3], **8**, 3 (1926) [*C.A.*, **22**, 3656 (1928)].
8) O. Hesse, *Ann. Chem.*, **271**, 214 (1892).
9) A.J. Ultée, *Pharm. Weekblad*, **61**, 1118 (1924) [*C.A.*, **19**, 474 (1925)].
10) A.J. Ultée, *Bull. Jardin Botan. Buitenzorg* [3], **5**, 241 (1922) [*C.A.* **18**, 2083 (1924)].
11) O. Hesse, *Ann. Chem.*, **271**, 180 (1892).
12) S. Iseda, *Kagaku No Ryoiki*, **11**, 208 (1957).
13) All melting points were measured on microscopic hotsage and not corrected.

Isolation of α-Amyrin Palmitate——Hexane extract (Fr. I, 54 g) was chromatographed on alumina (600 g) and eluted as follows.

(a) hexane	2000 ml	amorphous solid
(b) hexane–ether (1:1)	1200 ml	orange red oil
(c) ether	2000 ml	crystalline solid

Fraction (a) was not further studied. Fraction (b, 8.5 g) was rechromatographed on alumina and the elution with hexane gave viscous and colorless oil (2.5 g). This oil was chromatographed on silica gel (100 g) and eluted with hexane–benzene (9:1). The first eluate (500 ml) was removed, then evaporation of solvent from the following eluate gave a waxy solid containing prisms (0.9 g), which was rechromatographed on silica gel with the same solvent and recrystallized from EtOH–ether to yield prisms or needles (320 mg), mp 73—74°, $[\alpha]_D^{23}$ +52.5° (c=1.0, benzene), showing positive (purple) Liebermann–Burchard reaction. *Anal.* Calcd. for $C_{46}H_{80}O_2$: C, 83.06; H, 12.13. Found: C, 82.95; H, 11.82. IR ν_{max}^{KBr} cm^{-1}: 1730 (C=O), 1175 (CO–O).

Hydrolysis of α-Amyrin Palmitate——The crystals (200 mg) were refluxed with 30 ml of 5% alcoholic KOH for five hours. After cooling and removing EtOH *in vacuo*, water was added to the residue, the solution was extracted with ether and evaporation of ether afforded crystalline residue which was recrystallized from EtOH to give colorless needles (105 mg), mp 180°, identical with authentic α-amyrin by mixed melting point and comparison of their IR spectra. *Anal.* Calcd. for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.12; H, 11.97. IR ν_{max}^{KBr} cm^{-1}: 3300 (OH). The crystals were acetylated with acetic anhydride–pyridine and the acetate was recrystallized from EtOH to give colorless needles, mp 223—224°, which were identified with α-amyrin acetate by mixed melting point and comparison of their IR spectra. *Anal.* Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 82.01; H, 11.09. IR ν_{max}^{KBr} cm^{-1}: 1735 (C=O).

The alkaline solution after removing α-amyrin was neutralized with dilute HCl and extracted with ether. After removal of ether the residue was recrystallized from MeOH to yield colorless leaflets (46 mg), mp 60—61°, which were identified with authentic palmitic acid by mixed melting point and comparison of their IR spectra. *Anal.* Calcd. for $C_{16}H_{32}O_2$: C, 74.94; H, 12.58. Found: C, 75.10; H, 12.38. IR ν_{max}^{KBr} cm^{-1}: 1705 (C=O). In addition the methyl ester of the acid was compared with methyl palmitate by GLC and gave a single peak having the same retention time as the authentic sample. Hitachi Gas Chromatograph (KGL–2E) equipped with thermal conductivity detector was used under the following condition: Column (1.8 m) packed with Gas chrom–P coated with 13% EGS, temp. 195°; detector temp. 195°; sampler temp. 300°; carrier gas, He.

Identification of α-Amyrin Palmitate on TLC——In order to compare α-amyrin palmitate with synthesized sample by TLC it was prepared as follows. α-Amyrin (20 mg) was dissolved in pyridine (2 ml) and palmitoyl chloride (60 mg) was added into this solution, then the reaction mixture was warmed in water bath for few minutes and followed to stand overnight. This mixture was used as a sample solution to spot on the same chromatoplate (Silica Gel G, Merk) together with α-amyrin palmitate and developed with hexane–benzene (5:1). After drying the plate 10% H_2SO_4 was sprayed on the plate, which was heated until spots appeared. α-Amyrin plamitate had a single brown spot (*Rf* 0.58) which subsequently changed to purple and also the sample solution gave the same spot in *Rf* value and color reaction as α-amyrin palmitate with unreacted α-amyrin, palmitic acid and other few spots.

Isolation of Phytosterol——The crystalline solid obtained from fraction (c) was recrystallized from EtOH to give β-sitosterol as colorless plates (190 mg), mp 138°. *Anal.* Calcd. for $C_{29}H_{50}O$: C, 83.99; H, 12.15. Found: C, 83.62; H, 12.12. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH). The crystals were acetylated with acetic anhydride–pyridine and the acetate was recrystallized from EtOH to give colorless needles, mp 124°. *Anal.* Calcd. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48. Found: C, 81.55; H, 11.22. IR ν_{max}^{KBr} cm^{-1}: 1735 (C=O). This crystal and its acetate were identified with β-sitosterol and its acetate by mixed melting point but in comparison of IR spectra it was found that the spectrum has a small peak at 970 cm^{-1} based on stigmasterol in addition to that of β-sitosterol. GLC was carried out to examine the purity of β-sitosterol and comparison with the retention times of authentic samples clarified that it contained small amount of stigmasterol and less amount of campesterol. Shimadzu Gas Chromatograph (GC–1B) equipped with hydrogen flame detector was used under the following condition: Column (3 m) packed with Chromosorb Wcoated with 1.5 % SE–30, temp. 240°; sampler temp. 284°; detector temp. 265°; carrier gas, N_2.

Isolation of Ursolic Acid——Removal of solvent from ether extract (Fr. II) left dark green residue (20 g), which was extracted with hot MeOH and filtered. The filtrate was evaporated, dissolved in ether again and extracted with 10% KOH solution. The needles which appeared in the boundary face between ether and alkaline solution were collected, washed with ether, acidified with dilute H_2SO_4 and extracted with ether. Evaporation of ether afforded the crystalline residue which was recrystallized from EtOH to obtain colorless needles (225 mg), mp 278—279°, and identified with authentic ursolic acid by mixed melting point and comparison of their IR spectra. *Anal.* Calcd. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.59. Found: C, 78.17; H, 10.46. IR ν_{max}^{KBr} cm^{-1}: 3475 (OH), 1710 (C=O). The crystals were acetylated with acetic anhydride–pyridine and the acetate was recrystallized from EtOH to give colorless needles, mp 280—281°, which were identified with ursolic acid acetate by mixed melting point and comparison of their IR spectra. *Anal.* Calcd. for $C_{32}H_{50}O_4$: C, 77.06; H, 10.11. Found: C, 76.82; H, 10.07. IR ν_{max}^{KBr} cm^{-1}: 1730 (C=O).

The water insoluble residue (Fr. IV) was extracted with hot MeOH, the extract was filtered and removal of solvent from the filtrate gave dark brown and crystalline substance which yielded further ursolic acid (100 mg) as colorless needles with repeated recrystallization from EtOH.

Isolation of Potassium Nitrate——To the water soluble fraction (Fr. III) 30% lead acetate solution and subsequently saturated solution of basic lead acetate were added and excess lead was removed with H_2S from the filtrate. Concentration of the filtrate *in vacuo* separated a large amount of crystalline substance, probably more than 10 g, which was recrystallized from water to give colorless prisms (1.9 g), mp 330°. The crystals were an inorganic compound, giving the reactions of potassium and nitrate, and identified with potassium nitrate.

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