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Forsythia Koreana NAKAI씨(토연교)의 성분에 관한 연구(III). Ursolic acid의 분리 및 확인

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(1973. 7. 17 접수)

Studies on the Components of Fruits of Forsythia Koreana NAKAI(III). Occurrence of Ursolic Acid in the Fruits of Forsythia Koreana

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요 약. Forsythia Koreana NAKAI씨를 methanol로 추출분리하여 얻은 sapogenin 및 그유도체들을 mass spectra, ultraviolet spectra, infrared spectra, n.m.r spectra 원소분석, 정성실험으로 확인한 결과 ursolic acid임이 밝혀졌다.

Abstract. From the methanol extract of the fruits of Forsythia Koreana NAKAI a sapogenin and its derivatives were isolated. Through the mass spectra, ultraviolet spectra, nuclear magnetic resonance spectra, elemental analysis and chemical tests it was identified as ursolic acid.

Introduction

Forsythia Koreana NAKAI is a plant which is widely distributed all over the Korean Peninsula. The fruits are used as a medicine effective for the lowering of fever and the dissolving of blood clots. In 1938, Kunimine and Suzuki[1] isolated forsythin ($C_{27}H_{34}O_{11}$) from the stem of Forsythia Koreana. In 1947 A. Sosa[2] isolated phillyrim ($C_{27}H_{34}O_{11}$) which is identical with Forsythin from the stem of Forsythia Suspensa as well as four different unknown crystalline nonglycoside substances from the ether extract. Three of these four substances gave positive Lieberman test.

In 1953[3] A. Sosa and V. Plouvier succeeded in separating eight crystalline substances from the flowers of Forsythia Suspensa. In 1954, W. K.Koo and M.L. King[4] separated sapogenin and flavonol glycoside from the fruits of Forsythia

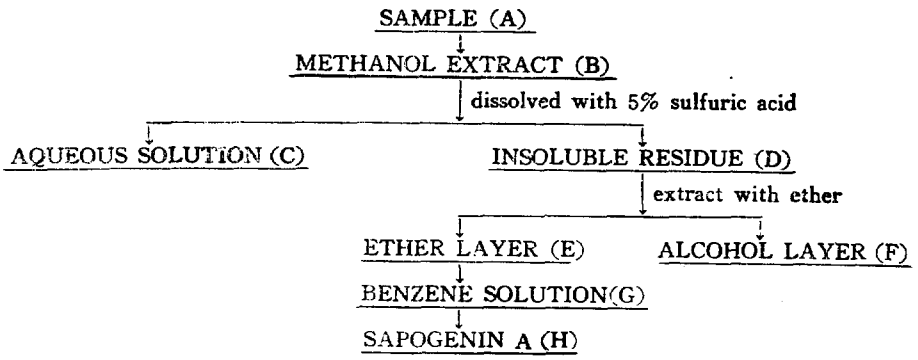


Fig. 1. The scheme of separation and purification of sapogenin.

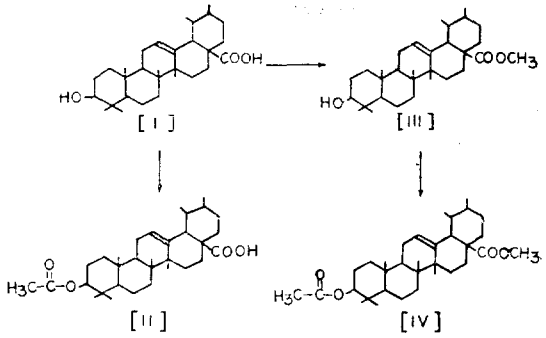
Suspensa and identified them by the color reaction.

In 1960, Fujita, Hisamichi[5] and his co-workers separated the pigment constituent, flavonal glycoside, from the flower of Forsythia Koreana. Consequently, the stem and fruits of Forsythia Koreana have not yet been thoroughly examined for their constituents except for the separation of forsythin by Kunimine.

The authors of this paper have already reported about the separation of some phytosterols, sapogenins, flavonol glycosides and a quaternary base from the fruits of Forsythia Koreana[6] and about the identification of quaternary base as betaine hydrochloride[7]. In this paper we report that sapogenin *A* was identified as ursolic acid by separation and identification through the physical and chemical method. *Fig.*1 shows the scheme of separation and purification of sapogenin *A* from the sample (crushed fruit of Forsythia Koreana).

Discussion

The molecular weight of purified sapogenin was found to be 456 through the mass spectrum. The molecular formula was determined to be $C_{30}H_{48}O_3$ by elemental analysis. The molecular weight of sapogenin *A* acetate was found to be 498, and the molecular formula $C_{32}H_{50}O_4$.

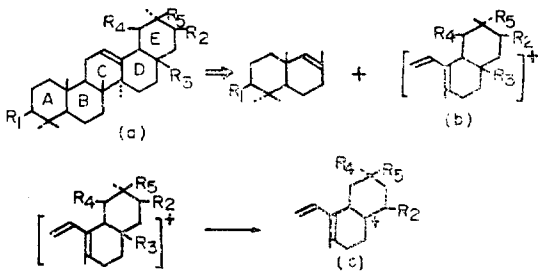


Therefore one can conclude that this is a monocetate. Since sapogenin *A* gives positive Lieberman test, negative Tschugaev test, and has the above characteristics, it must belong to the triterpene family. The presence of n.m.r. signal at τ=4.76(*Fig.* 4) and a positive Baeyer test indicates the presence of carbon-carbon double bonds. Although the IR spectra(*Fig.* 2) showed that there was an -OH group(3410 cm^{-1}) and a $-\overset{O}{\overset{\|}{C}}-$ group (1685 cm^{-1}), the IR spectra of acetate did not show an—OH group. Therefore, one of the three oxygen in this sapogenin *A* was an alcoholic —OH group. When 5% sodium hydroxide solution was added into the ether solution of the sapogenin, a white precipitate was obtained. This is redissolved into ether by the acidification with 5 % sulfuric acid solution. Since it gives a negative 2,4-dinitrophenyl-

hydrazine test, the carbonyl group on the IR spectra is neither an aldehyde nor a ketone but an acid. This was also conformed through the formation of a methyl ester with diazomethane (see experiment III). Ordinarily carboxylic acid gives a broad peak at 3,000 cm^{-1} in the IR spectra but *Fig.* 2 shows a sharp peak. This might be due to the fact the $-COOH$ in this case is at highly hindered position such as at a tertiary carbon atom. The shift of proton signal due to the $-COOH$ group toward higher field, could also be understood from the same reason.

Especially, the fragmentation pattern appearing in the mass spectra of sapogenin and its derivatives pointed strongly to such a Δ^{12}-amyrin skeleton (see *Fig.* 3). It has recently been established[9,10] that the molecular ion of compounds of the type (*a*) undergoes a reverse Diels-Alder fragmentation to furnish a characteristic ion (*b*), corresponding to ring *D* and *E*. The peak (*c*) ion is generally followed by a second peak corresponding to (*b*) minus the C-17 substituent.

The mass spectra of the sapogenin showed



very strong peaks at *m/e* 248 and 203. This was highly reminiscent of the fragmentation pattern[11] of methyl siaresinolate 3-acetate (R_1=AcO, R_3=$COOCH_3$, R_4=OH, R_2=R_5=H), which showed a strong m/e 278 peak (*b* ion), followed by peaks at *m/e* 260 (*b* − H_2O) and *m/e* 201 (*b* − H_2O − R_2). If it is assumed that the sapogenin has a amyrin skeleton with R_3=COOH, R_1=OH, R_4=CH_3 and R_5=H, the peak at *m/e* 248 would be assigned as (*b*) and the prominent peak at *m/e* 203 as *b* − R_3.

Species (*c*) suffers further decomposition by the loss of 70 mass units yielding a fragmenta tion. This cleavage is probably due to the partial

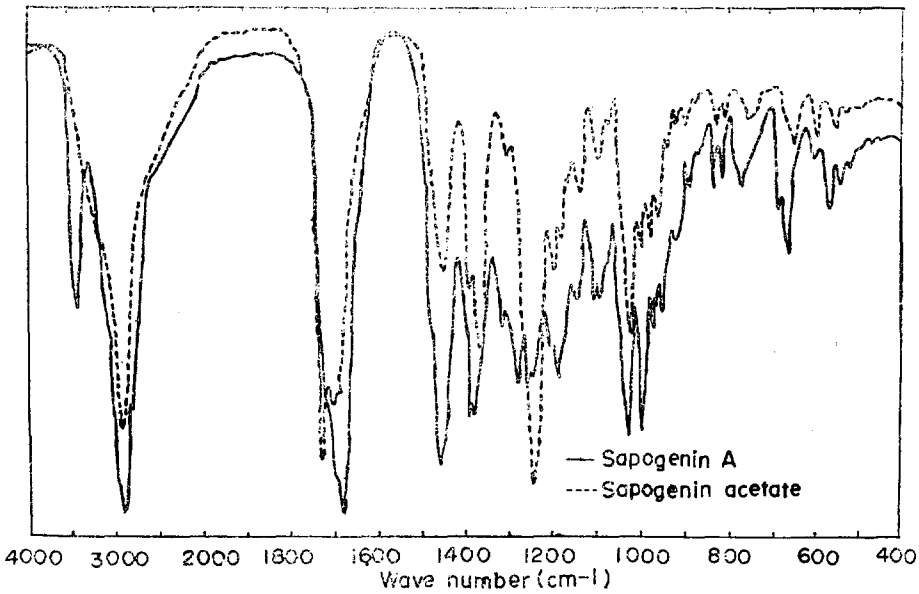


Fig. 2. Infrared spectra of sapogenin *A* and sapogenin acetate.

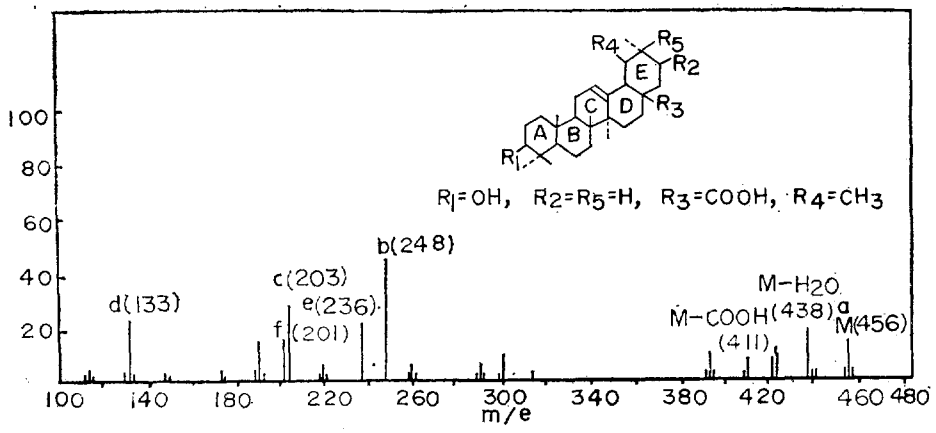


Fig. 3. Mass spectrum of sapogenin.

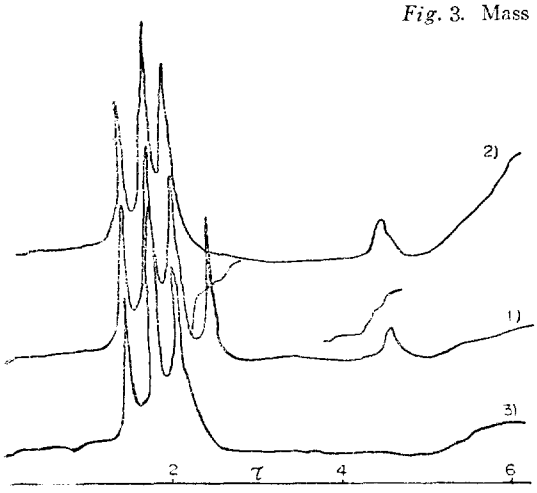
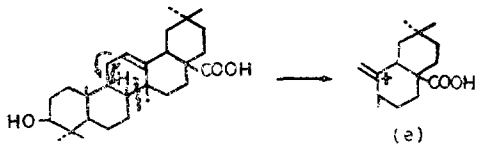


Fig. 4. NMR spectra of sapogenin *A* and its derivative. 1)sapogenin, 2)methyl ester, 3)DMF.

loss of ring *E* yielding the highly stabilized ion (*d*):

Species (*b*) is accompanied by a fragment (*e. g.* m/e 236) of relatively low abundance and containing 13 mass units less than (*b*) ion. For its genesis, the following process seems probably to involve one hydrogen transfer and cleavage of an allylically activated bond (yielding species (*e*)):



The fragmentation of (*f*) must involve transfer of one hydrogen atom and the following mechanism can be proposed:

Species (*f*) is one of the most characteristic fragmentation products of saturated pentacyclic triterpenes.

All these indications give strong support for an amyrin type skeleton and the location of carboxyl group at C-17. It is logical to suppose that the hydroxyl group is of the 3β-type as in most triterpenes, since the n.m.r signal at $\tau=6.7$ is assumed due to α-axial proton at C-3(*Fig.* 5). Therefore, sapogenin *A* could be excluded a Δ^{12}-α-amyrin ($R_4=H$, $R_5=CH_3$) or Δ^{12}-β-amyrin ($R_5=H$, $R_4=CH_3$). If sapogenin *A* had included α-amyrin series such as oleanolic acid where the double bond is known to be more reactive than in β-amyrin it would have reacted

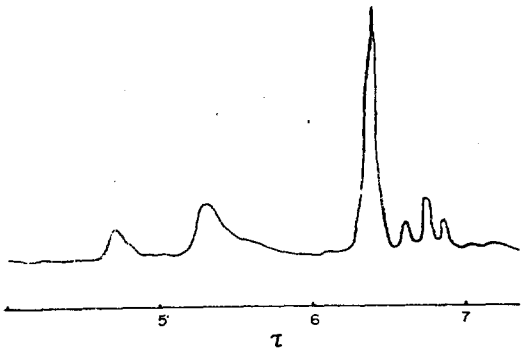
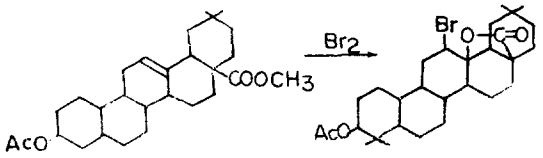


Fig. 5. NMR spectrum of sapogenin methyl ester. (TMS; as an internal standard)

instantaneously with bromine to form 12α-bromo γ-lactone as follows:[12]



However sapogenin *A* did not react appreciably even after 24 hours. Indeed, other chemical proof of the structure of sapogenin *A* such hydrogenation[12], showed that it belongs to the β-amyrin series.

Experiment

The Separation and Purification of Sapogenin *A* (I). The sample, 10 kg(*A*), was crushed and extracted three times by warm methanol, and then this extract was concentrated under reduced pressure. This concentrated extract (*B*) was treated with 5 % sulfuric acid and separated acid solution (*C*) from the insoluble residue (*D*). The residue (*D*) was dissolved in a small amount of methanol and extracted by ether in a liq.-liq. extractor. The ether solution (*E*) was evaporated and the residue was added in benzene (*G*). The precipitate was filtered, washed well with acetone and dried. It was then dissolved in methanol and run through active alumina to remove resin and tar. The solution was then treated with active charcoal and concentrated under reduced pressure. Upon standing, a crystalline precipitate separated out. Several recrystalizations from methanol afforded pure sapogenin *A* (*H*), m.p., 283—285 °C (lit[8]., 285 °C for ursolic acid). *Anal.* Calcd. for $C_{30}H_{48}O_3$: C, 78.95 ; H, 10.75. Found: C, 78.37 ; H, 10.88. This crystalline substance is slightly solube in ether and acetone but is much more soluble in methanol. Purified sapogenin *A* (0.2—0.3 mg) was dissolved In 0.5 m*l* of anhydrous acetic acid. When a drop of concentrated sulfuric acid was added, a purple color appeared which gradually changed to blue and finally red at the boarder line of the two liquid phase (Liebermann Burchard Reaction). Another color change was observed from yellow to red after 30 minutes when *conc.* sulfuric acid was added. When a methanol solution of the same sample was treated with 1-2 drops of 15% ethanol solution of α-naphthol and 1m*l* of *conc.* sulfuric acid no red color observed between two layers (Molishtest). These results lead to the conclusion that this is a nonglycoside sapogenin. It was negative to the Tschugaev test.

Sapogenin *A* Acetate (II). Purified sapogenin *A* (3 gr) was mixed with 35 m*l* of pyridine and 15 m*l* of acetic anhydride at room temperature and the solution was left overnight. The mixture was poured on ice and the precipitate was collected. The precipitate was recrystalized in methanol several times. White needlelike crystals, sapogenin *A* acetate (II), m.p., 288 °C (lit[8]., 289 °C for ursolic acid acetate) were obtained. *Anal.* Calcd. for $C_{32}H_{50}O_4$: C, 77.42 ; H, 10.09. Found: C, 76.61 ; H, 10.44.

Sapogenin *A* Methyl Ester (III). A sample (100 mg) of the acid (I) was suspended in 8 m*l*. of ether and an ethereal solution of approximately 50 mg of diazomethane was added. The mixture was left overnight, and then evaporated. Recrystallization of the residue from chlo-

roform-methanol afforded methyl ester(III) m.p., 169 °C(lit[8]., 170—171 °C for methyl ursolate). *Anal.* Calcd. for $C_{31}H_{50}O_3$: C, 79.48 ; H, 10.69. Found: C, 78.62 ; H, 11.12.

Sapogenin *A* Methyl Ester Acetate(IV). Acetylation of 50 mg of sapogenin *A* methyl ester(III) was affected overnight with acetic anhydride-pyridine at room temperature. The mixture was poured on ice, and the precipitate was collected. Recrystallization from methanol-chloroform, afforded methylester acetate(IV), m.p., 245 °C (lit[8]., 246—247 °C for methyl ursolate acetate) *Anal.* Calcd. for $C_{33}H_{52}O_4$: C, 77.85 ; H, 10.19. Found:C, 78.46 ; H, 10.56.

Conclusion

From the fruits of Forsythia Koreana NAKAI sapogenin A was separated and purified. Colorless crystals m.p., 283∼285 °C were obtained. The sapogenin derivatives were synthesized.

Through the mass spectra and elementary analysis, molecular weights and formulas were determined. Further, by ultraviolet spectra infrared spectra, n.m.r spectra and chemical reactions, this compound was identified as ursolic acid.

Acknowledgment

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