[Chem. Pharm. Bull. 15(11)1682~1686(1967)]

UDC 581.19 : 547.597

214. Eiichi Fujita, Tetsuro Fujita, and Toyoko Suzuki*1 : On the Constituents of *Nauclea orientalis* L.*2 I. Noreugenin and Naucleoside, A New Glycoside. (Terpenoids V*3)

(*Institute for Chemical Research, Kyoto University**1)

β-Sitosterol, noreugenin (I), palmitic acid, and naucleoside (VII), a new triterpene glycoside, have been isolated from *Nauclea orientalis* L. The direct isolation of 2-methyl-5,7-dihydroxychromone(noreugenin) (I) from natural source has never been published, although it has been synthesized and also derived from eugenin (II) by demethylation.

Upon acidic hydrolysis, naucleoside gave quinovaic acid (III) as the aglycon, together with D-xylose and L-rhamnose as the sugar moiety. The order of linkage is shown to be represented by D-xylose-L-rhamnose-(C-3)-quinovaic acid (VII).

(Received December 12, 1966)

Nauclea orientalis L. (Japanese name "Taniwatari-no-ki") (Rubiaceae) grows in the southern part of Kyushu in Japan. The present paper deals with the isolation of some components and their constitution.

Ethereal extract from the heart wood of the plant on separation and purification gave as crystals a neutral substance, a phenolic compound, an acid, and a triterpene glycoside.

The neutral crystal proved to be identical with β-sitosterol by comparison of itself and its acetate with authentic samples respectively.

A compound from phenolic fraction was obtained as colorless needles, $C_{10}H_8O_4$, m.p. 268° (decomp.), exhibiting a positive Gibbs' reaction.[1] The infrared (IR) spectrum suggested the presence of hydroxyl groups (3400~2600 cm^{-1}) and an α,β-unsaturated carbonyl group (1660 and 1620 cm^{-1} [KBr]), and the ultraviolet spectrum was characteristic of chromone series giving the absorption maxima at 227, 249, 256 and 295 mμ. In the nuclear magnetic resonance (NMR) spectrum*6 taken in dimethylsulfoxide, a couple of doublets (J=2 c.p.s.) appeared at δ 6.32 and 6.20 p.p.m. which were assigned to two protons in meta relationship on a benzene ring. The hydroxyl proton signals appeared as singlets at δ 12.8 and 10.8 p.p.m. The paramagnetic shift of the former is due to a hydrogen bond with carbonyl which exists near the hydroxyl group. Another one proton signal on a double bond was observed as a quartet (J=0.7 c.p.s.) at δ 6.15 p.p.m., while in the NMR spectrum taken in pyridine a doublet (J=0.7 c.p.s.) assigned to methyl protons on a double bond appeared at δ 2.13 p.p.m.

From the foregoing spectral data, the structure 2-methyl-5,7-dihydroxychromone (I) was assigned to the phenolic substance. Although compound I has not yet been found in nature, it has been obtained by Schmid, *et al.*[2] through demethylation of eugenin (II), and its total synthesis has been accomplished by Jochum, *et al.*[3]. A comparison of the phenol with authentic 2-methyl-5,7-dihydroxychromone (I) established their identity. The monomethyl ether of the phenol also proved to be identical with eugenin

*1 Takatsuki, Osaka-fu (藤田栄一, 藤多哲朗, 鈴木豊子).

*2 A part of this paper was presented at the annual meeting of Kinki Branch of the Pharmaceutical Society of Japan held at Kobe on Nov. 20th, 1965.

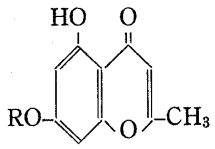
*3 Part IV. Tetrahedron Letters, **1966**, 3153.

1) H.D. Gibbs : J. Biol. Chem., **72**, 649 (1927); H. Inouye, Y. Kanaya, Y. Murata : This Bulletin, **7**, 573 (1959).

2) Th. M. Meijer, H. Schmid : Helv. Chim. Acta, **31**, 1603 (1948).

3) E. Jochum, St. v. Kostanecki : Ber., **37**, 2099 (1904).

(Ⅱ) by a direct comparison with the authentic sample. Hence, we propose the name "noreugenin" to this phenol.



I : R=H
Ⅱ : R=CH_3

Chart 1.

An acidic component was isolated as crystals, m.p. 61~64°. The IR spectrum of the compound was superimposable with that of authentic palmitic acid. The gas-liquid chromatography (g.l.c.) of the methyl ester confirmed that it is identical with methyl palmitate.

The initial ethereal extract was concentrated to give a precipitate, which was purified by recrystallization from a mixture of methanol and ethyl acetate to yield crystals, $C_{41}H_{64}O_{13}\cdot 1\frac{1}{2}H_2O$, m.p. 260~261°. The latter gave a positive Liebermann-Burchard reaction, and its acid hydrolysate gave a positive Fehling test. We propose the name "naucleoside" to this new glycoside. The hydrolysis by heating with 10% ethanolic hydrochloric acid for 9 hours afforded an aglycon as crystals, $C_{30}H_{46}O_5$, m.p. 294~295° (decomp.), which gave a positive tetranitromethane test and exhibited yellow color with Bromocresol Green indicator. The IR spectrum (KBr) had the absorption bands at 3550 (OH), 1690 and 1680 cm^{-1} (COOH). Methylation of the aglycon with diazomethane gave a neutral dimethyl ester, $C_{32}H_{50}O_5$, m.p. 179.5~181°, whose IR spectrum still had the absorption band due to a hydroxyl group. The ester on acetylation gave a monoacetate as crystals, $C_{34}H_{52}O_6$, m.p. 214~216°, whose NMR spectrum showed the presence of two methoxyl (δ 3.61 and 3.63 p.p.m.), one acetyl (δ 2.05 p.p.m.), and six C-methyl (near δ 0.83 p.p.m.) groups and of a hydrogen on a carbon attached to an acetoxyl group (quartet at δ 4.50 p.p.m., J=9.2, 6.5 c.p.s.) and one trisubstituted double bond (1H, triplet, δ 5.65 p.p.m., J=3.0 c.p.s.).

Based on the foregoing data, the aglycon proved to have two carboxyl groups, one secondary hydroxyl group, and one trisubstituted double bond in the molecule, hence to be a pentacyclic triterpene hydroxydicarboxylic acid. Moreover, the physical constants of the aglycon and its derivatives seemed very similar to those of quinovaic acid (Ⅲ).[4,5] an aglycon from quinovin in *Cortex Chinae*, and its derivatives. The comparison of the aglycon with the authentic sample of quinovaic acid (Ⅲ) confirmed their complete identity. Dimethyl ester (Ⅳ), dimethyl ester acetate (Ⅴ) and acetate (Ⅵ) were also compared and identified with the corresponding derivatives, respectively.

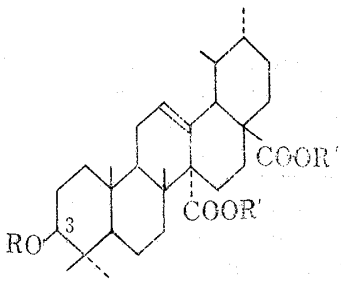


Chart 2.

Ⅲ : R=R′=H
Ⅳ : R=H; R′=CH_3
Ⅴ : R=CH_3CO; R′=CH_3
Ⅵ : R=CH_3CO; R′=H
Ⅶ : R=D-Xylose-L-Rhamnose-; R′=H

The mother liquor free from the aglycon after acidic hydrolysis was evaporated to give a residue, whose paper chromatogram under the conditions described in the experimental part gave two spots; one of them, colored in red with aniline hydrogen phthalide,[6] was judged as an aldopentose, while the other one, colored in grayish brown with the same reagent, was judged as a methylpentose. Their Rf values 0.34 and 0.42 were identical with those of xylose and rhamnose respectively. Naucleoside on acetylation yielded a pentaacetate, $C_{51}H_{74}O_{18}$, m.p. 244~245° (decomp.), whose NMR spectrum

4) J. Simonsen and W.C.J. Ross : "The Terpenes" Vol. V, p. 75 (1957). University Press, Cambridge.
5) R. Tschesche, I. Duphorn, G. Snatzke : Ann., **667**, 151 (1963).
6) S.M. Partridge : Nature, **164**, 443 (1949).

gave methyl proton signals equivalent to five acetyl groups at δ 1.98~2.23 p.p.m. Thus, it is clear that the sugar portion of naucleoside consists of a pentose and a methylpentose and they are linked to the aglycon with its hydroxyl group at C-3, but with neither of its carboxyl groups.

Subsequently, a partial hydrolysis of the glycoside under weaker conditions[7] was tried in order to determine the sequence of the sugar linkage. In earlier times, only a spot of pentose was observed on the thin-layer chromatogram using crystalline cellulose (Abisel), but after 15 hours, both spots of pentose and methylpentose appeared. These observations well explain the order of the linkage such as aglycon-methylpentose-pentose.

Finally, it was confirmed by the following experiments that the methylpentose is L-rhamnose, and the pentose is D-xylose. The sugar portion which was made free from the aglycon after complete hydrolysis of naucleoside was chromatographed on a cellulose powder column.[5,8] The methylpentose fraction possessing Rf value of 0.42, which appeared as one spot on a paper chromatogram, gave $[\alpha]_D^{23}$ +8.6°, which coinsided with the value ($[\alpha]_D^{20}$ +8.9°) of L-rhamnose.*4 Another pentose fraction possessing Rf value of 0.34, which also proved its homogeneity on a paper chromatogram, gave $[\alpha]_D^{28}$ +10.7°.*5 The methylpentose was converted to a tetratrimethylsilyl ether[9] by its treatment with hexamethyldisilazane and trimethylchlorosilane in pyridine. The g.l.c. of the ether under the conditions described in the experimental part gave two peaks, and their retention times were identical with those of two peaks (α- and β-) which appeared on the gas chromatogram of the tetratrimethylsilyl ether of authentic L-rhamnose. The mixed g.l.c. again exhibited two peaks possessing the original retention times. Thus, the methylpentose in naucleoside proved to be L-rhamnose.

The quite same treatments on the pentose gave two peaks on the gas chromatogram and they were confirmed as those of ethers of D-α-xylose and D-β-xylose by direct comparison with authentic sample. Thus, the structure of naucleoside can be represented by D-xylose-L-rhamnose-quinovaic acid (Ⅶ).

The studies on the more detailed mode of sugar linkage in naucleoside and on the other glycosides in the same plant source are in progress.

Experimental*6

Extraction from *Nauclea orientalis* L.——Dried heart wood (3.5 kg.) was cut in small pieces and extracted with ether at room temperature for 4 days. Ethereal extract was concentrated to give a precipitate (D) (560 mg.), and the filtrate was extracted with 5% NaOH aq.. The alkaline layer was made acidic to give a precipitate, which was extracted with ether. The latter ethereal extract was separated into soluble and insoluble fractions in $NaHCO_3$ aq.. The usual treatment yielded crude phenolic substance(B) (3.6 g.) and crude $NaHCO_3$ soluble substance (C) (3.9 g.). The mother ethereal solution from the extraction with 5% NaOH aq. was washed with dil. HCl, followed by the usual treatment to give crude neutral substance(A) (5.1 g.).

β-Sitosterol——Column chromatography on neutral alumina of neutral substance(A) was effected to isolate a compound as crystals (600 mg.), when eluted with $CHCl_3$-C_6H_6(1:4). It was purified by recrystallization from acetone to yield colorless needles, m.p. 139.5~140°, $[\alpha]_D^{18}$ −33.7° (c=0.6, $CHCl_3$), giving a positive Liebermann-Burchard reaction, which proved to be identical with the authentic β-sitosterol by mixture melting point determination and comparison of their IR spectra. IR cm^{-1} : ν_{O-H} 3620($CHCl_3$). *Anal.* Calcd. for $C_{29}H_{50}O \cdot {}^1/_2H_2O$: C, 82.26; H, 12.06. Found : C, 82.12; H, 12.16. The crystals (58 mg.) were allowed to stand overnight in the mixture of pyridine (2 ml.) and Ac_2O (2 ml.). After evaporation *in vacuo* to dryness, the residue was purified by recrystallization from MeOH-$CHCl_3$ to yield colorless needles, m.p. 192°, which

*4 The aq. solution is at first levorotatory (−7°), but in a few minutes becomes dextrorotatory (+8.9°).
*5 cf. D-xylose : $[\alpha]_D^{20}$ initial +92°, final +19°.
*6 All melting points were uncorrected. Nuclear magnetic resonance spectra were taken with a Varian A-60 Spectrometer using tetramethylsilane as an internal standard.

7) R.J. Morris, E.W. Hussey : J. Org. Chem., **30**, 166 (1965).
8) T. Takemoto, K. Kometani : Ann., **685**, 237 (1965).
9) H.E. Brower, J.E. Jeffery, M.W. Folsom : Anal. Chem., **38**, 362 (1966).

was identified with β-sitosterol acetate by mixture melting point determination and IR comparison. IR cm^{-1} : $\nu_{C=O}$ 1720. *Anal.* Calcd. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48. Found : C, 81.37; H, 11.30.

Noreugenin (I)——Phenolic fraction(B) was easily crystallized. Recrystallization from MeOH gave colorless needles, m.p. 268° (decomp.), whose Gibbs' reaction was positive. UV λ_{max}^{MeOH} mμ (log ε) : 227(4.08), 249(4.13), 256(4.13), 295(3.65). IR cm^{-1} : ν_{O-H} 3400~2600, $\nu_{C=C-C=O}$ 1660, 1620(KBr). NMR $\delta_{p.p.m.}^{DMSO}$: 6.15 (1H, quartet, J=0.7 c.p.s., $\underline{H}>C=C<CH_3$), 6.20(1H, doublet, J=2.0 c.p.s.), 6.32(1H, doublet, J=2.0 c.p.s.), 10.8(1H, singlet, -O$\underline{H}$), 12.8(1H, singlet, -O$\underline{H}$); $\delta_{p.p.m.}^{pyr.}$: 2.13(3H, doublet, J=0.7, c.p.s. $\underline{H}>C=C<\underline{CH_3}$). The IR spectrum was completely identical with that of the authentic sample of 2-methyl-5,7-dihydroxychromone (I), m.p. 265~269° (decomp.). *Anal.* Calcd. for $C_{10}H_8O_4$:C, 62.50; H, 4.20. Found : C, 62.67; H, 4.63.

Methylation of noreugenin : Noreugenin (100 mg.) on methylation with CH_2N_2 in Et_2O-MeOH and purification of the crude product by column chromatography on silica gel followed by recrystallization from EtOH gave needles, m.p. 118°. On admixture with the authentic eugenin, m.p. 118°, the melting point was not depressed and their IR spectra were completely superimposable. The Rf values on silica gel thin-layer chromatogram (MeOH-$CHCl_3$; 1:9) were completely identical.

Palmitic Acid——The elution of the acidic fraction(C) on a silica gel column with C_6H_6-$CHCl_3$ (1:1) gave a compound as crystals, m.p. 53~54°, which was recrystallized from MeOH to yield needles, m.p. 61~64°. IR cm^{-1} : $\nu_{C=O}$ 1700($CHCl_3$). NMR $\delta_{p.p.m.}^{CDCl_3}$: 0.89(3H, triplet, J=5.0 c.p.s., $-CH_2-C\underline{H}_3$). 1.28 (singlet), 2.35 (2H, triplet, J=8.0 c.p.s.). The compound was identified with palmitic acid by mixture melting point determination and IR comparison. The methyl ester was compared and identified with methyl palmitate by g.l.c.. A Hitachi Perkin-Elmer Gaschromatograph F 6 equipped with a hydrogen flame detector was used. The column was a stainless steel tube (2.00 m. × 3 mm. in diameter) packed with Chromosorb W coated with 2% polyethyleneglycol adipate ester. Separation was operated at 200° with N_2 gas pressure of 2.0 kg./cm^2. The retention times of our sample and authentic methyl palmitate were 2.1 min., and the mixed sample also gave a single peak possessing the same retention time.

Naucleoside (VII)——The precipitate(D) was crystallized from MeOH-AcOEt, and purified by recrystallization to naucleoside, m.p. 260~261°. The Liebermann-Burchard reaction was shown to be positive with the latter. *Anal.* Calcd. for $C_{41}H_{64}O_{13}\cdot 1^1/_2H_2O$: C, 62.20; H, 8.47. Found : C, 62.38; H, 8.57. Pentaacetate : Naucleoside(54 mg.) was dissolved in pyridine(2 ml.) and Ac_2O(2 ml.), and the solution was allowed to stand overnight at room temperature. The crude product obtained by usual treatment of the reaction mixture was purified by recrystallization from MeOH to give a pentaacetate as needles, m.p. 244~245° (decomp.). IR cm^{-1} : $\nu_{C=O}$ 1695, 1760; ν_{O-H} 3400; δ_{C-O} 1230(KBr). NMR $\delta_{p.p.m.}^{pyr.}$: 1.98(3H, singlet), 2.05(6H, singlet), 2.13(3H, singlet), 2.23(3H, singlet). *Anal.* Calcd. for $C_{51}H_{74}O_{18}$: C, 62.83; H, 7.59. Found : C, 62.83; H, 7.89.

Hydrolysis of Naucleoside i) Quinovaic Acid (III)——Naucleoside(100 mg.) was mixed with conc. HCl (3 ml.), water(1 ml.) and EtOH(6 ml.), and the mixture was heated on a boiling water bath for 9 hours. After cooling, the precipitate(50 mg.) was gathered by filtration and purified by recrystallization from EtOH to give an aglycon as crystals, m.p. 294~295°(decomp.), $[\alpha]_D^{18}$ +90° (c=1.9, 5% KOH), which gave yellow color to tetranitromethane and Bromocresol Green indicator, respectively. IR cm^{-1} : ν_{O-H} 3550; $\nu_{C=O}$ 1680, 1690(KBr). NMR $\delta_{p.p.m.}^{pyr.}$: 0.88(3H, doublet), 0.93(3H, singlet), 0.99(3H, singlet), 1.10(3H, singlet), 1.15(3H, singlet), 1.20(3H, doublet). *Anal.* Calcd. for $C_{30}H_{46}O_5$: C, 74.03; H, 9.53;. Found : C, 74.39; H, 9.80. The IR spectrum was superimposable with that of the authentic sample of quinovaic acid. Methylation with CH_2N_2 gave dimethyl ester(Ⅳ), m.p. 179.5~181°. The melting point was not depressed, when mixed with the authentic sample of quinovaic acid dimethyl ester, m.p. 178°. *Anal.* Calcd. for $C_{32}H_{50}O_5$: C, 74.70; H, 9.72. Found : C, 74.79; H, 9.84. The aglycon on acetylation gave a monoacetate (Ⅵ), m.p. 281~284° (decomp.) (from CH_3CN-MeOH). Dimethyl ester acetate(Ⅴ) which was obtained from the aglycon by methylation with CH_2N_2 followed by acetylation with Ac_2O-pyr. had m.p. 214~216°(from MeOH). NMR $\delta_{p.p.m.}^{CDCl_3}$: near 0.83(6×C-$C\underline{H}_3$), 2.05(3H, singlet, $C\underline{H}_3CO$), 3.61(3H, singlet, $OC\underline{H}_3$), 3.63(3H, singlet, $OC\underline{H}_3$), 4.50 (1H, quartet, J=9.2, 6.5 c.p.s., $-CH_2-C\underline{H}<\overset{OAc}{C<}$), 5.65(1H, triplet, J=3.0 c.p.s., $-C\underline{H}_2>C=C<$). On admixture with the authentic quinovaic acid dimethyl ester acetate, m.p. 208~209°, the melting point was not depressed. *Anal.* Calcd. for $C_{34}H_{52}O_6$: C, 73.34; H, 9.41. Found : C, 73.29; H, 9.52.

ii) Rhamnose and Xylose——The mother liquor after removing the aglycon was evaporated to dryness. The residue was paper chromatographed on Toyo Roshi No. 50 using the upper layer of *n*-BuOH-AcOH-H_2O (4:1:5) as solvent and detected by aniline hydrogenphthalate.[6] One red spot giving Rf 0.34 and another grayish brown spot giving Rf 0.42 appeared.

Naucleoside(96 mg.) was heated on a water-bath(78°) with 95% EtOH(4.5 ml.), H_2O(2.5 ml.) and conc. HCl (0.05 ml.). After 7 minutes, the clear solution (only a small sample) was neutralized with 0.01*N* NaOH, and evaporated to dryness. The thin-layer chromatogram of the residue on crystalline cellulose using the same solvent and reagent as in paper chromatography gave only one red spot possessing the same Rf value with that of xylose. The similar treatments after 15, 55, and 95 minutes gave the same spot. After 15 hours, however, another grayish brown spot whose Rf value was identical with that of rhamnose appeared.

Subsequently, a mixture of naucleoside(535 mg.), MeOH(10 ml.), and conc. HCl(10 ml.) was heated at 75～80° for 12 hours. The mother liquor after separating the precipitate(311 mg.) was washed with AcOEt, and the aqueous layer was passed through Amberlite IRA-410 to remove acid. Evaporation of the solution gave a sugar mixture(84 mg.). The latter(60 mg.) was chromatographed on a cellulose powder column(5 g.); the column was packed using the under layer of *n*-BuOH-AcOH-H_2O(4:1:5), while the elution was carried out using the upper layer of the same mixtrue. Consequently, a fraction(A), which gave $[\alpha]_D^{23}$ +8.6°(c=1.0, H_2O) and proved to be homogeneous on paper chromatogram(Rf=0.42), and another fraction(B), which gave $[\alpha]_D^{23}$ +10.7°(c=0.7, H_2O) and also exhibited one spot on paper chromatogram(Rf=0.34), were clearly separated. A very small quantity of the former fraction(A) was mixed into a solution of hexamethyldisilazane (0.2 ml.), trimethylchlorosilane(0.1 ml.), and pyridine(1.0 ml.) and allowed to stand for 20 minutes. The excess reagent and solvent were evaporated by spraying N_2 gas to dryness. The resulting trimethylsilyl ether was taken up with acetone and gas chromatographed. A Shimadzu GC-IC Gas Chromatograph equipped with a hydrogen flame detector was used. The column was a glass tube(1.835 m. × 4 mm. in diameter) packed with Chromosorb W coated with 1.5% SE-30. Nitrogen was used as a carrier gas at a flow rate of 35 ml./min.. Column temperature was 130°. The retention times were as follows :

Our sample	:	13.4 and 18.4 min.
Authentic L-rhamnose tetratrimethylsilyl ether	:	13.4 and 18.4 min.

The quite same chromatogram was obtained also when both samples were mixed.

Quite similarly, a very small quantity of the latter fraction(B) was converted to a trimethylsilyl ether and gas chromatographed. The same apparatus was used under the foregoing conditions, provided that column temperature was 155°. The retention times of two peaks were as follows :

Our sample	:	5.2 and 6.6 min.
Authentic D-xylose tetratrimethylsilyl ether	:	5.2 ond 6.6 min.

We are indebted to Prof. S. Hatsushima, Kagoshima University, for the collection and discernment of the plant, and to Prof. H. Inouye, Kyoto University, Prof. H. Schmid, Chem. Inst. Zürich University, and Prof. R. Tschesche, Org.-Chem. Inst. University of Bonn, for the gifts of β-sitosterol, chromones, and quinovaic acid, respectively. We express our thanks to Dr. T. Shingu, Kyoto University, for NMR determination and discussion, and also to the members of the Microanalytical Center of Kyoto University for the analytical data.