Studies on the Medicinal Resources. XXXVI.¹⁾ The Constituents of the
Leaves of *Saxifraga stolonifera* MEERBURG (Saxifragaceae)²⁾

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Two flavonol glycosides, I and II, were isolated from the fresh leaves of *Saxifraga stolonifera* MEERBURG (Saxifragaceae). I is a new flavonol glucoside, $C_{21}H_{20}O_{12} \cdot H_2O$, mp 264°, and was named saxifragin. The structure of saxifragin (I) has been determined as quercetin-5- β -D-glucoside by chemical and spectral means.

II, pale yellow needles, mp 186—190°, was identified as quercitrin by direct comparison with an authentic sample.

Saxifraga stolonifera MEERBURG (Saxifragaceae) is a decorative and medicinal plant. Though the fresh leaves of the plant have been used for a burn, frostbite and whooping cough in Japanese folk medicine, the chemical component has not been reported.

We now wish to report a new flavonol glycoside to which we gave the name saxifragin (I) and isolation of a known flavonol glycoside (II) from the fresh leaves of *Saxifraga stolonifera* MEERBURG.

I, mp 264°, and II, mp 186—190°, have now been isolated from the ethyl acetate extract of the fresh leaves of this plant.

I corresponded to the molecular formula of $C_{21}H_{20}O_{12} \cdot H_2O$ by elemental analyses, was colored green to ferric chloride, showed bright yellow fluorescence under ultraviolet light (UVL), and exhibited a positive color test for flavonoids.

Ultraviolet (UV) absorption spectrum of I showed maxima at 256 nm (log ϵ 4.52) and 375 nm (log ϵ 4.53). Its infrared (IR) absorption spectrum indicated the presence of hydroxyl group, carbonyl group and double bond in the molecule.

Hydrolysis of I with 10% hydrochloric acid afforded an aglycone, melting at over 300°, in 60.5% yield, which was identified as quercetin (3,5,7,3',4'-pentahydroxyflavone) by direct comparison with the authentic specimen. Sugar portion was treated as usual, and the sugar was identified as D-glucose by paper partition chromatography (PPC), and its osazone, mp 205°. I gave an octaacetate, mp 247°, under usual acetylation.

In the nuclear magnetic resonance (NMR) spectrum of trimethylsilyl (TMS) ether of I, a broad signal integrating six protons at 3.2—4.1 ppm and a doublet (1H, $J=6.0$ Hz) at 5.18 ppm were assigned the aliphatic protons and the anomeric proton⁴⁾ of the sugar moiety, respectively.

Based on these facts mentioned above, I is quercetin- β -D-glucoside. Though quercetin-monoglucosides have been reported as 3-glucoside (isoquercitrin),⁵⁾ 7-glucoside (quercimeritrin),⁶⁾

1) Part XXXV: N. Morita, M. Shimizu, M. Arisawa and S. Kitanaka, *Yakugaku Zasshi*, **94**, 875 (1974).

2) The 37th Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, Toyama, October 1973.

3) Location; *Gofuku* 3190, Toyama.

4) T.J. Mabry, K.R. Markham and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, New York, N.Y., 1970, p. 261.

5) S. Hattori, *Nippon Kagaku Zasshi*, **58**, 844 (1937).

6) A.G. Perkin, *J. Chem. Soc.*, **95**, 2181 (1905).

4'-glucoside (spiraeoside)⁷⁾ and 3'-glucoside,⁸⁾ physical properties of I differ from those of quercetin-monoglucosides in Table I.

Oxidation of I with hydrogen peroxide afforded 3,4-dihydroxybenzoic acid.

In the UV spectral experiments of I, addition of both aluminum chloride and sodium acetate showed the bathochromic shifts. I gave a positive Zircon-citric acid test,⁹⁾ therefore the bathochromic shift in its UV spectrum by aluminum chloride will be due to hydroxyl group at 3-position.

When I-permethyleate was hydrolyzed on refluxing with 10% hydrochloric acid, 3,7,3',4'-tetramethoxy-5-hydroxyflavone (quercetin-3,7,3',4'-tetramethyl ether), mp 159°, was obtained and identified by direct comparison with an authentic specimen.

Consequently, saxifragin (I) is best represented as 3,5,7,3',4'-pentahydroxyflavone-5- β -D-glucoside (quercetin-5- β -D-glucoside).

II, pale yellow needles, mp 186—190°, was identified as quercitrin by direct comparison with an authentic specimen.

TABLE I. Comparison of Quercetin-Monoglucosides and Saxifragin (I)

	mp (°C)	Acetate mp (°C)
3-Glucoside (isoquercitrin)	248°	167—169° ^{a)}
7-Glucoside (quercimeritrin)	247—249°	216—217° ^{a)}
4'-Glucoside (spiraeoside)	210—212°	126—128° ^{a)}
3'-Glucoside	228—230°	169—170°
Saxifragin (I)	264°	247°

a) measured in this laboratory

Experimental¹⁰⁾

Isolation of Saxifragin (I)—10 kg of the fresh leaves of *Saxifraga stolonifera* MEERBURG (collected at Kamiichi-machi, Toyama, in June) were extracted with MeOH. Evaporation of methanol yielded aqueous extract. The extract was extracted with ether and then ethyl acetate. The combined ethyl acetate extract was concentrated to about a half of the original volume, and was allowed to stand overnight. The crude yellow crystals were obtained. Recrystallization from pyridine-MeOH afforded yellow micro-needles, 200 mg, mp 264°, green to FeCl₃. Mg+HCl; red, UVL; bright yellow fluorescence, Zircon-citric acid test; positive, Molish reac.; positive. *Anal.* Calcd. for C₂₁H₂₀O₁₂·H₂O: C, 52.26; H, 4.60. Found: C, 52.20; H, 4.66. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 256 (4.52), 308 (sh) (3.68), 375 (4.53). UV $\lambda_{\text{max}}^{\text{EtOH-AlCl}_3}$ nm: 264, 430. UV $\lambda_{\text{max}}^{\text{EtOH-AcONa}}$ nm: 257, 272, 315—328, 380. UV $\lambda_{\text{max}}^{\text{EtOH-AcONa-H}_3\text{BO}_3}$ nm: 258, 312, 389. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3200, 1620, 1600, 1560, 1510, 1415, 1370, 1335, 1315, 1275, 1205, 1105, 1075, 1045, 1030, 995, 925, 885, 850, 825, 790. NMR (TMS ether of I, 10% solution in CCl₄) δ (ppm): 3.2—4.1 (6H, broad, aliphatic H $\times$ 6), 5.18 (1H, doublet, J =6.0 Hz, anomeric H), 6.38 (1H, doublet, J =2.0 Hz, C₆-H), 6.51 (1H, doublet, J =2.0 Hz, C₈-H), 6.87 (1H, doublet, J =9.0 Hz, C₅-H), 7.64 (1H, doublet, J =2.0 Hz, C₂'-H), 7.70 (1H, quartet, J =2.0 Hz, J =9.0 Hz, C₆'-H).

Hydrolysis of Saxifragin (I) with 10% HCl—A solution of 100 mg of saxifragin (I) in 10% HCl was refluxed over an open flame for a half hr. The precipitated aglycone was collected, and was recrystallized from MeOH. Yellow needles, melting at over 300°, yielding 60.5 mg, greenish brown to FeCl₃ and yellow under UVL. IR spectrum of the aglycone was found to be superimposable with that of an authentic specimen of quercetin.

- 7) P. Casparis and E. Steinegger, *Pharm. Acta. Helv.*, **20**, 174 (1945); J.B. Harborne, *Experientia*, **17**, 72 (1961).
- 8) P.K. Denliev, Z.P. Pakudina and A.S. Sadykov, *Dokl. Akad. Nauk. USSR*, **20**, 19 (1963) [*Chem. Abstr.*, **60**, 10775 (1964)].
- 9) L. Härhammer and R. Hänsel, *Arch. Pharm.*, **286**, 425 (1953).
- 10) Melting points are uncorrected and were taken on a Mitamura micro melting point apparatus. IR and UV spectra were recorded on a Japan Spectroscopic Co., LTD. Spectrophotometer, Model IR-E, and on a Hitachi Spectrophotometer, Model 124, respectively. NMR spectrum was obtained on a Japan Electron Optics Lab., JNMC-60H. Chemical shifts were recorded as δ values (ppm) with TMS internal standard.

Acetylation of the aglycone with Ac_2O and pyridine in the usual manner gave pentaacetate, mp 247° , undepressed on admixture with an authentic quercetin pentaacetate.

After removal of the aglycone, the mother liquor was treated as usual. PPC *Rf*; 0.31 (*n*-BuOH-AcOH- H_2O (4:1:2), glucose 0.31), 0.38 (*n*-BuOH-pyridine- H_2O (6:4:3), glucose 0.38). Color reaction with 0.1*N* aniline hydrogen phthalate; reddish brown. The osazone was formed as yellow needles, mp 205° , undepressed on admixture with glucosazone, mp 207° .

Saxifragin Octaacetate—Saxifragin (I) dissolved in acetic anhydride in the presence of a few drops of conc. H_2SO_4 was allowed to stand over night at room temperature. After the usual work-up, crystallization from MeOH gave colorless needles, mp 247° , no color to FeCl_3 . *Anal.* Calcd. for $\text{C}_{37}\text{H}_{36}\text{O}_{20}$: C, 55.48; H, 4.53. Found: C, 55.59; H, 4.64.

Methylation of Saxifragin (I)—A suspension of saxifragin (I) (100 mg) in acetone (30 ml) was refluxed for 48 hr with K_2CO_3 (2 g) and dimethyl sulfate (1.5 ml), and poured into ice-water. The precipitate was collected, washed with H_2O and dried. Colorless powder, mp $162\text{--}166^\circ$, no color to FeCl_3 .

Quercetin-3,7,3',4'-tetramethyl Ether—A solution of saxifragin permethylate in 10% HCl was refluxed over an open flame for a half hr. The precipitate was collected and was recrystallized from 80% MeOH to give yellow needles, mp 159° , purplish brown to FeCl_3 . Zircon-citric acid test; negative. *Anal.* Calcd. for $\text{C}_{19}\text{H}_{18}\text{O}_7$: C, 63.66; H, 5.07. Found: C, 63.80; H, 5.11. Its IR spectrum was found to be hardly distinguishable from that of the authentic specimen, and it was undepressed on admixture with an authentic sample.

Isolation of Quercitrin (II)—The filtrate of saxifragin (I) was provided to PPC (40 cm $\times$ 40 cm, solv.; *n*-BuOH-AcOH- H_2O (4:1:2)), and separated band (*Rf* 0.8) was cut off and extracted with MeOH. The MeOH extract was purified with silica gel column (solvent; CHCl_3 -MeOH (5:1)), and recrystallization from MeOH gave pale yellow needles, mp $186\text{--}190^\circ$. Its IR spectrum was found to be hardly distinguishable from that of the authentic specimen, and it was undepressed on admixture with an authentic sample.

Hydrolysis of Quercitrin (II)—A solution of 100 mg of quercitrin (II) in 10% HCl was refluxed over an open flame for a half hr. The aglycone was recrystallized from MeOH. Yellow needles, melting at over 300° . IR spectrum of the aglycone was found to be superimposable with that of an authentic specimen.

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