34% yield from **4d**), mp 254—257° (dec.) (lit.[21] mp 249—251°); UV (Table II); IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3385, 3280 (b), 3205 (b), 3095 ($NH_2$, $CONH_2$), 1663, 1632 ($CONH_2$, C=N), 1556 ($CONH_2$); NMR ($Me_2SO$-$d_6$) $\delta$: 5.05 (2H, s, $CH_2Ph$), 5.78 (2H, dull s, $NH_2$), 6.64 (2H, dull s, $CONH_2$),[20] 7.08—7.36 (6H, Ph, imidazole proton). *Anal.* Calcd. for $C_{11}H_{12}N_4O$: C, 61.09; H, 5.59; N, 25.91. Found: C, 60.81; H, 5.61; N, 25.92.

**5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamide (7j)**——A mixture of conc. aq. $NH_3$ (8 ml) and crude **5j**·HCl, prepared from **4f** (290 mg, 0.8 mmol) according to the procedure described under the synthesis of **5j**, was heated in a sealed tube at 100° for 16 hr. A small amount of an insoluble material was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in $H_2O$ (5 ml) and a saturated solution (15 ml) of picric acid in $H_2O$ was added. The yellow precipitate (269 mg) that resulted was filtered off, washed with a little $H_2O$, and dissolved in $H_2O$ (400 ml). The aqueous solution was passed through a column of Amberlite IRA-402 ($HCO_3^-$) (4.5 ml). Elution with $H_2O$ and evaporation of the eluate (500 ml) *in vacuo* left crude **7j** (155 mg), mp 207—209° (dec.). Recrystallization from 90% (v/v) aq. EtOH gave almost colorless prisms (98 mg, 47% yield from **4f**), mp 213—214.5° (dec.) (lit. mp 213—214°;[22] mp 218°[23]); UV (Table II). This sample was identical (by PPC, mixed melting-point test, and IR spectrum) with an authentic sample of AICA riboside.

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# Studies on the Constituents of Useful Plants. VI.[1] Constituents of the Calyx of *Diospyros kaki* (2), and Carbon-13 Nuclear Magnetic Resonance Spectra of Flavonol Glycosides

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Structure of substances XIV and XVIII, obtained previously among the constituents of the calyx of persimmons (*Diospyros kaki*, Ebenaceae), was examined, and XIV was determined as astragalin (kaempferol 3-β-D-glucopyranoside) and XVIII as *n*-butyl-β-D-fructopyranoside, although the latter was considered to be an artifact. Carbon-13 NMR spectra of kaempferol, quercetin, trifolin, astragalin, and hyperin were measured and their structure was proved from the assignment of NMR signals.

**Keywords**——Ebenaceae; *Diospyros kaki* Thunb.; astragalin; hyperin; trifolin; *n*-butyl-β-D-fructopyranoside; carbon-13 nuclear magnetic resonance spectra

Our previous report[3] described the isolation of higher fatty acids, aromatic acids, flavonols and their glycosides, steroids, and triterpenoids as a constituent of the calyx of persimmons (*Diospyros kaki*, Ebenaceae). In the present series of work, structure of substances XIV

1) A part of this work was presented at the Osaka Meeting of Japanese Pharmacognostic Society, October 1974 and at the 96th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya 1976.
2) Location: *Mitahora-Higashi, Gifu, 502, Japan.*
3) S. Matsuura and M. Iinuma, *Yakugaku Zasshi*, **97**, 452 (1977).

and XVIII, among the constituents of the said calyx, was elucidated, and carbon-13 nuclear magnetic resonance ($^{13}$C-NMR) spectra of flavonoids were examined.

Substance XIV was obtained as yellow crystals, mp 212—214°. It was positive to the ferric chloride, magnesium–hydrochloric acid, zinc–hydrochloric acid, and Molish–Udransky reactions, and gave kaempferol and glucose on hydrolysis. The position of the linkage of the sugar was considered to be the 3-position of kaempferol from the ultraviolet (UV) absorption spectrum and UV spectra of XIV after addition of various reagents. Consequently, XIV was assumed to be kaempferol 3-monoglucoside (astragalin) but the melting point of astragalin has been variously reported as 178°,[4] 182—185°,[5] 197—198°,[6] 223°,[7] and 225°,[8] and the sample of astragalin we obtained melted at 224°. Therefore, peracetate of XIV was prepared and its NMR spectrum was measured, in which the signals for four $CH_3$ protons due to the acetoxyl bonded to the sugar were observed at 1.95—2.16 ppm, and those of three $CH_3$ protons due to the phenolic acetoxyl at 2.39—2.49 ppm. Therefore, this glucoside was established as kaempferol 3-monoglucoside, and identified with the authentic sample of astragalin.[7]

The crystalline substance XVIII of mp 149—151° was positive to the Molish–Udransky and Keller–Killiani reactions, suggesting that it is a sugar derivative. Its composition corresponded to $(C_5H_{10}O_3)_n$ from its elemental analytical values and its mass spectrum of *m/e* 236 for $M^+$ indicated that the composition would be $C_{10}H_{20}O_6$. Hydrolysis of XVIII with sulfulic acid produced fructose, and XVIII was assumed to be the fructoside of $C_4H_9OH$. Infrared (IR) spectrum of XVIII exhibited absorptions due to the tetrahydropyran ring at 865 and 780 $cm^{-1}$,[9] besides absorptions for OH, $CH_3$, and $CH_2$, suggesting that the fructose is a pyranose, not furanose. It is known that, when fructose takes a furanose-type conformation, a stable fragment ion of furan structure is produced by the liberation of a substituent at C-2, followed by cleavage between C-5 and C-6,[10] whereas if it is a pyranose type, only the liberation of a substituent at C-2 occurs and a stable fragment ion pyran structure appears.[11] (In the case of pentaacetate of $\beta$-D-fructopyranose, *m/e* 331 for $M^+-O\cdot COCH_3$). The mass spectrum of the peracetate of XVIII showed the fragment ion formed by liberation of the butoxyl group at *m/e* 331 ($M^+-OC_4H_9$), followed by the fragment ions at *m/e* 211, 169, and 109, formed by the rearrangement of the acetoxyl group.[12] This was an evidence that XVIII is a pyranose-type fructoside.

In general, molecular optical rotation of glycosides is less affected by the substituents, compared to their anomers, according to Hudson's rule for equioptical rotation.[13] The optical rotation of XVIII, $[\alpha]_D-138°$, is close to that of methyl-$\beta$-D-fructopyranoside[14] of −173° ($\alpha$-type, $[\alpha]_D+80°$). Further, in $^{13}$C-NMR (in $(CD_3)_2SO$) spectrum of XVIII, signals due to $\beta$-, $\gamma$-, and $\delta$-carbons[15] appear respectively at 31.8, 18.9, and 13.4 ppm, and signals of fructose carbons at 100.0, 69.3, 68.9, 63.7, 62.1, and 59.4 ppm. The chemical shift of these signals for fructose carbons agree more with that of fructopyranose than that of fructofuranose.[16]


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These evidences suggest that XVIII is *n*-butyl-*β*-D-fructopyranoside, and it was found to be identical with an authentic specimen of *n*-butyl-*β*-D-fructoside synthesized by the reaction of *β*-D-fructose and *n*-butanol at room temperature, in the presence of a dehydrating agent.[17] However, there has been no example of the presence of a *n*-butyl-glycoside in nature and it was assumed that XVIII is an artifact formed during the process of extraction by the condensation of fructose contained in the crude drug with *n*-butanol used for extraction of the constituents.

In recent years, $^{13}$C-NMR spectrometry is being used increasingly for the structural determination of flavonoids. Kingsbury and Looker[18] measured $^{13}$C-NMR of flavone (A) (in $CDCl_3$) and assigned the signals as shown in Table I.

On the other hand, Stothers[19] proposed the additivity with respect to the chemical shift produced by the introduction of a substituent in the benzene ring, and stated that, when the substituent was a hydroxyl, signal of the carbon into which OH group was substituted shift to a lower field by 27 ppm (*β*-effect) and that of the two carbon atoms *ortho* to the carbon atom substituted with OH group shifted to a higher field by 12.6 ppm. The $^{13}$C-NMR spectra of kaempferol (B) and quercetin (C)[20] were measured (in $(CD_3)_2SO$) and the signals were assigned according to Stothers' additive rule (*cf.* Table I).

These $^{13}$C-NMR data of kaempferol and quercetin were in good agreement with the assignment for 3′,4′,5,7-tetramethoxyflavonol (D) (in $CDCl_3$) made by Kingsbury and

Table I. $^{13}$C Chemical Shifts of Flavone (A), Kaempferol (B), Trifolin (E) Astragaline (F), and Hyperin (G)

| | | A[a] | B | E | F | G |
|---|---|---|---|---|---|---|
| Aglycone moieties | C-2 | 131.5, 163.0[b] | 146.7 | 156.4 | 156.3 | 156.3 |
| | 3 | 107.3 | 135.7 | 133.4 | 133.1 | 133.3 |
| | 4 | 178.0 | 175.8 | 177.5 | 177.4 | 177.4 |
| | 5 | 128.4 | 156.2 | 156.4 | 156.3 | 156.3 |
| | 6 | 124.9 | 98.2 | 98.8 | 98.7 | 98.8 |
| | 7 | 133.5 | 163.8 | 164.2 | 164.1 | 164.1 |
| | 8 | 117.9 | 93.4 | 93.8 | 93.6 | 93.6 |
| | 9 | 156.0 | 160.7 | 161.1 | 161.1 | 161.1 |
| | 10 | 123.7 | 103.0 | 104.0 | 104.0 | 104.0 |
| | 1′ | 131.5, 163.0[b] | 121.7 | 120.9 | 120.9 | 121.6 |
| | 2′ | 126.0 | 129.5 | 131.0 | 130.8 | 116.2 |
| | 3′ | 128.8 | 115.4 | 115.1 | 115.0 | 144.8 |
| | 4′ | 131.3 | 159.2 | 159.9 | 159.8 | 148.4 |
| | 5′ | 128.8 | 115.4 | 115.1 | 115.0 | 115.3 |
| | 6′ | 126.0 | 129.5 | 131.0 | 130.8 | 116.2 |
| Sugar moieties | 1 | | | 101.9 | 100.8 | 101.0 |
| | 2 | | | 71.3 | 74.1 | 74.1 |
| | 3 | | | 73.1 | 77.3 | 77.4 |
| | 4 | | | 68.0 | 69.8 | 69.9 |
| | 5 | | | 75.7 | 76.3 | 76.4 |
| | 6 | | | 60.3 | 60.8 | 61.0 |

Except A all spectra were measured in $(CD_3)_2SO$.
*a*) In $CDCl_3$. *b*) Tentative assignment only. These pairs may be interchanged.

17) Org. Syntheses, Collected Vol. **1**, 364.
18) C.A. Kingsbury and J.H. Looker, *J. Org. Chem.*, **40**, 1120 (1975).
19) J.B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press Inc., New York, 1972, p. 196.
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Looker.[18] This fact indicates that the structure of hydroyflavones can be assigned from their $^{13}$C-NMR *per se*, without methylation of their hydroxyl groups, and is a valuable experimental result in the structural elucidation of flavonoids.

These $^{13}$C-NMR data were then used to determine the kind and position of the sugar attached to flavonols by application to flavonoids. The signal patterns of trifolin and astragalin[21] (Fig. 1) agree respectively with the overlapped signals of kaempferol and sugar portion of methyl-β-D-galactopyranoside[22] and those of kaempferol and sugar portion of methyl-β-D-glucopyranoside,[22] except for the shift of the signal at 135.7 ppm (C-3) in kaempferol to a higher field by about 2.6 ppm, and a shift of the signal at 146.7 ppm (C-2) to a lower field by about 10 ppm to overlap with the signal of carbon at 5-position in kaempferol at 156.3 ppm.

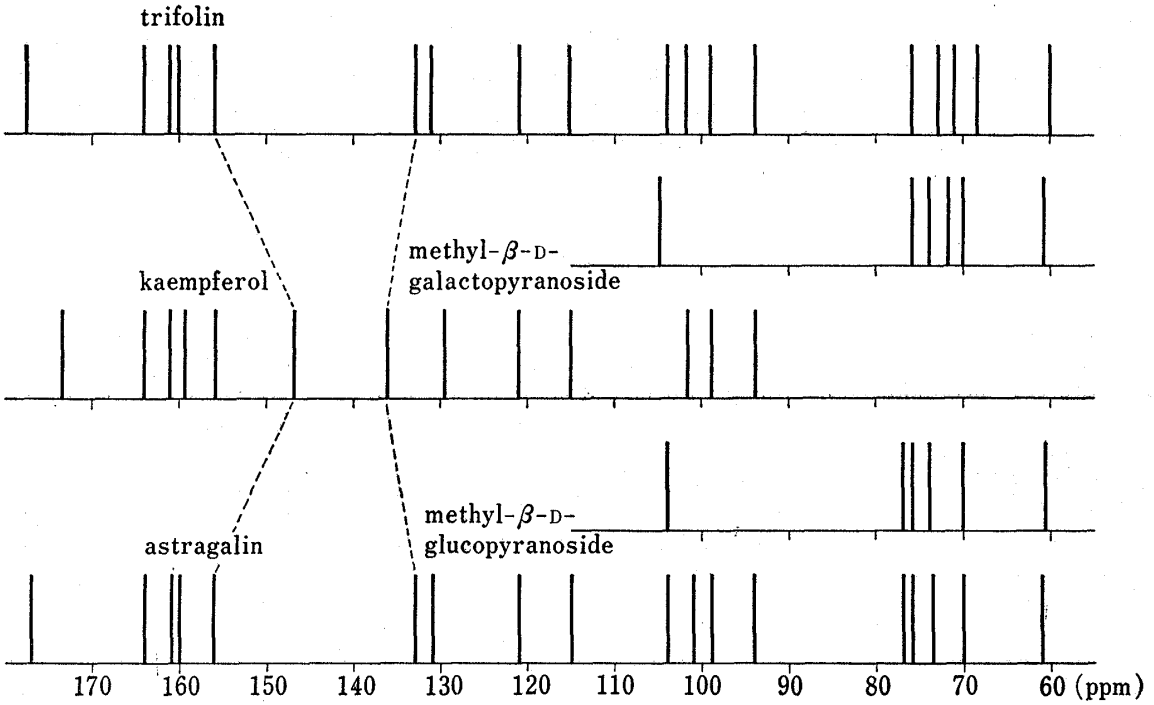


Fig. 1. Correlation of the $^{13}$C-NMR Spectra of Trifolin, Kaempferol and Astragalin

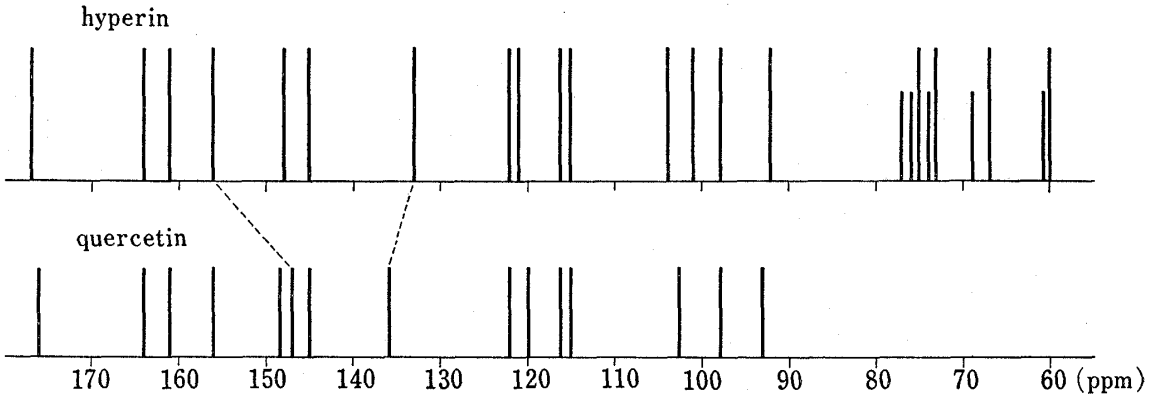


Fig. 2. Correlation of the $^{13}$C-NMR Spectra of Hyperin and Quercetin

21) N. Morita, M. Arisawa, M. Nagase, H.Y. Hsu, and Y.P. Chen, *Yakugaku Zasshi*, **97**, 649 (1977).
22) P.A.J. Gorid and M. Mazurek, *Can. J. Chem.*, **53**, 1212 (1975).

These results indicate that trifolin is kaempferol 3-$\beta$-D-galactopyranoside and that astragalin is kaempferol 3-$\beta$-D-glucopyranoside.

The signal pattern of hyperin (Fig. 2) agrees completely with that of quercetin except for the shift of the signal of carbon at 3-position in quercetin to a higher field by about 2.6 ppm, anda shift of the signal of carbon in 2-position to a lower field by about 10 ppm, thereby overlapping with that of carbon at 5-position, same as trifolin. In the signals of the sugar moiety, however, there are signals of methyl-$\beta$-D-glucopyranoside[22] besides those of methyl-$\beta$-D-galactopyranoside,[22] although that of the former is weaker than that of the latter, and the content must be small. This is in accordance with the fact that sugars other than D-galactose or other glycosides were not detected in paper chromatography of the sugar in the hydrolysate of hyperin.[3] Consequently, hyperin isolated in the present work is proved to be quercetin 3-$\beta$-D-galactopyranoside, and a small amount of quercetin 3-$\beta$-D-glucopyranoside is contaminated in this hyperin.

Thus, the structure of substances XIV and XVIII, obtained as the constituent in the calyx of *kaki*, was determined as astragalin and *n*-butyl-$\beta$-D-fructopyranoside. At the same time $^{13}$C-NMR spectra of flavonol glycosides (trifolin, astragalin, and hyperin) were measured to determine the kind, position, and manner of bonding of the sugar to flavonols in these compounds.

## Experimental

All melting points are uncorrected. Toyo Roshi No. 50 was used for the paper chromatography and Kieselgel G (Merck) for thin-layer chromatography (TLC). The coloring agent was 10% $H_2SO_4$ for TLC, and 3% $Mg(OAc)_2$ for flavonoids and aniline hydrogen phthalate for sugar in paper chromatography. IR spectra were taken with JASCO Model IRA-1, NMR with Hitachi-Perkin-Elmer (60 MHz), mass spectra (MS) with Hitachi RMU-6D, and UV with Shimadzu Model MPS-50L.

**Astragalin (XIV)**——XIV was obtained as pale yellow needles (from MeOH), mp 212—214°, 110 mg. Zn–HCl reaction, orange; Molish–Udransky reaction, positive; zircon–citric acid reaction, negative. Identified by mixed fusion with authentic astragalin, mp 223°,[7] and comparison of IR spectra and paper chromatograms. UV $\lambda_{max}^{MeOH}$ nm: 267, 300 (sh), 352; $\lambda_{max}^{AcONa}$ nm: 276, 307, 370; $\lambda_{max}^{AlCl_3}$ nm: 269, 295 (sh), 306, 352, 398; $\lambda_{max}^{AlCl_3/HCl}$ nm: 276, 296 (sh), 304, 347, 398; $\lambda_{max}^{MeONa}$ nm: 275, 327, 400. Paper chromatography: *Rf* 0.70 (astragalin, *Rf* 0.70) (15% AcOH). Astragalin peracetate; mp 212—214° (MeOH). NMR (in $CDCl_3$) $\delta$: 1.95—2.16 (sugar, –OC–C$\underline{H}_3\times4$), 2.39—2.49 (phenolic –CO–C$\underline{H}_3\times3$), 4.00, 5.05—5.40 (each 3H, br, aliphatic H, on glucose H), 5.53 (1H, d, $J$=6.0 Hz, anomeric $\underline{H}$), 6.90 (1H, d, $J$=2.0 Hz, C(6)-$\underline{H}$), 7.30 (2H, d, $J$=8.7 Hz, C(3′,5′)-$\underline{H}$), 7.44 (1H, d, $J$=2.0 Hz, C(8)-$\underline{H}$), 8.13 (2H, d, $J$=8.7 Hz, C(2′,6′)-$\underline{H}$).

Hydrolysis of Astragalin: A mixture of 20 mg of astragalin and 10 ml of 5% $H_2SO_4$ was heated for 2 hr, cooled, and yellow crystals that precipitated out were collected and recrystallized from AcOEt to the aglycone, mp 272—273°. The aglycone was identified with kaempferol by mixed fusion and paper chromatography. *Rf* 0.47 (60% AcOH), 0.16 (15% AcOH). Mother liquor of hydrolysis, left after collection of aglycone crystals, was neutralized with $BaCO_3$ and submitted to paper chromatography, from which the sugar was identified as glucose. *Rf* 0.38 (80% phenol) (glucose, *Rf* 0.38).

***n*-Butyl-$\beta$-D-fructopyranoside (XVIII)**——XVIII was obtained as colorless needles (from AcOEt), mp 149—150°, 20 mg. Molish–Udransky reaction, positive; Keller–Killiani reaction, positive. $[\alpha]_D^{21}$ −138° (MeOH). *Anal.* Calcd. for $C_{10}H_{20}O_6$: C, 50.74; H, 8.43. Found: C, 50.83; H, 8.53. MS, *m/e*: 236 ($M^+$), 205, 149, 103, 77. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 2950, 1400, 1120, 1060, 912, 890, 865, 780, 605. NMR ($CD_3OD$) $\delta$: 0.95 (3H, t, $-CH_2-C\underline{H}_3$), 1.51 (4H, br, $-C\underline{H}_2-C\underline{H}_2-$), 3.55 (2H, t, $-O-C\underline{H}_2$), 3.35—3.92 (7H).

XVIII was identified with *n*-butyl-$\beta$-D-fructoside, synthesized by the route shown below, by mixed fusion and comparison of IR spectra and thin-layer chromatograms. TLC: *Rf* 0.66 ($CHCl_3$–MeOH=5:1) (*n*-butyl-$\beta$-D-fructoside, *Rf* 0.66).

Peracetate: MS, *m/e*: 404 ($M^+$), 331, 275, 233, 211, 170, 149, 126, 109. NMR ($CDCl_3$) $\delta$: 0.96 (3H, t, $-CH_2-C\underline{H}_3$), 1.38 (4H, br, $-C\underline{H}_2-C\underline{H}_2-$), 1.99—2.18 (12H, each s, $4\times CO-C\underline{H}_3$), 3.57 (2H, $-O-C\underline{H}_2-CH_2-$), 3.89 (2H, C(1)-$C\underline{H}_2$), 4.22 (2H, C(6)-$\underline{H}$), 5.40 (3H, C(3,4,5)-$\underline{H}$).

Hydrolysis of *n*-Butyl-$\beta$-D-fructopyranoside: A mixture of 20 mg of *n*-butyl-$\beta$-D-fructopyranoside and 10 ml of 5% $H_2SO_4$ was heated for 2 hr, neutralized with $BaCO_3$, and fructose was identified from the hydrolysate solution by paper chromatography. *Rf* 0.31 (80% phenol) (fructose, *Rf* 0.31).

**Synthesis of *n*-Butyl-$\beta$-D-fructoside**——A solution of 5 g of dried fructose dissolved in 50 ml of 0.5% HCl in *n*-BuOH was stirred at room temperature for 30 hr. The reaction mixture evaporated to dryness and

the residue was purified by chromatography (developed with $CHCl_3$–MeOH (5: 1)) over a silica gel column, to give 1.0 g of a product with mp 149—151° (from AcOEt). TLC: *Rf* 0.66 ($CHCl_3$–MeOH=5: 1).

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