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The Constituents of *Arthraxon hispidus Makino, Miscanthus tinctorius Hackel, Miscanthus sinensis Anderss,* and *Phragmites communis Trinius*

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From the leaves and stems of *Arthraxon hispidus Makino*, aconitic acid, luteolin, luteolin-7-glucoside, and a new flavone, arthraxin, have been isolated. Luteolin, luteolin-7-glucoside, and arthraxin were also isolated from *Miscanthus tinctorius Hackel*, and tricin was isolated from *Miscanthus sinensis Anderss* and *Phragmites communis Trinius*.

Arthraxon hispidus Makino, called 'Kobunagusa,' in Japanese is widely distributed in Japan. This grass has been used as a source of natural dyestuff for coloring 'Kihachijo,' which is a traditional yellow silk cloth produced on Hachijo Island, Japan. As was stated in our previous paper,[1] a report on a chemical study of the dyestuff has been made by Hayashi *et al.*,[2] but without obtaining a pure material; they presumed that the yellow pigment of the grass was a glucoside of luteolin or quercetin. *Miscanthus tinctorius Hackel*, *Miscanthus sinensis Anderss*, and *Phragmites communis Trinius*, called 'Kariyasu,' 'Susuki,' and 'Yoshi' in Japanese respectively, have also been used as sources of natural yellow dyestuff.

Since no report on chemical studies of the coloring substance have been found in the literature, such a study has been undertaken by the present authors.

Arthraxin hispidus Makino

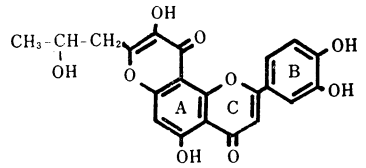
From the extract of the grass, the following four compounds, **1**—**4**, have been isolated. The methods of isolation for each of these compounds will be described in detail in the Experimental section.

*Arthraxin (**1**).* This substance is a new flavone named arthraxin, the structural formula of which **1** was described in a previous arthraxin paper.[1]

*Aconitic Acid (**2**).* This substance showed an acidic reaction with several reagents. Its analytical, IR and R_f values agreed with those of authentic

1) M. Kaneta and N. Sugiyama, This Bulletin, **42**, 2084 (1969): *J. Chem. Soc., C*, **1971**, 1982.

2) K. Hayashi and T. Inoue, *Acta Phytochimica* **15**, 53 (1949).



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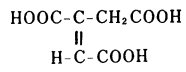

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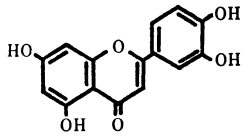

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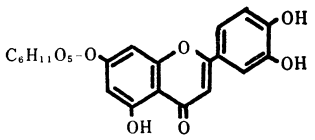

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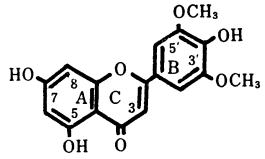


(5)

aconitic acid, and a mixed-melting-point determination with the authentic substance did not show any depression.

*Luteolin (**3**).* This substance showed a positive color reaction of flavone, and its analytical values agreed with those of the $C_{15}H_{10}O_6$ formula. On fusion with caustic potash, it yielded protocatechuic acid and phloroglucinol. The agreement of its melting point (325°C), R_f values, and UV spectra with those of luteolin[2-5] further supported its structure.

*Luteolin-7-glucoside (**4**).* This substance showed a positive color reaction of flavone, and its analytical values agreed with the $C_{21}H_{20}O_{11}$ formula. The hydrolysis of this compound produced luteolin and glucose. Since the UV λ_{max} of the II band did not shift significantly upon the addition of anhydrous acetate, the sugar was presumed to be attached to the 7-position of the aglycone. Thus, compound (**4**) was considered to be luteolin-7-glucoside. The final identification was based on the agreement of its melting point (255°C), R_f values, and UV spectra with those of luteolin-7-glucoside.[5-7]

3) T. Nakaoki, *J. Pharm. Soc. Japan*, **69**, 70 (1949).
4) T. A. Geissman, "The Chemistry of Flavonoid Compounds," Pergamon Press, Oxford, (1962), p. 111.
5) L. Jurd, *Arch. Biochem. Biophys.*, **63**, 376 (1956).

Miscanthus tinctorius Hackel

A preliminary examination of the ethanol extract of the grass by tlc and ppc showed the presence of almost the same flavonoid compounds as in *Arthraxon hispidus Makino*.

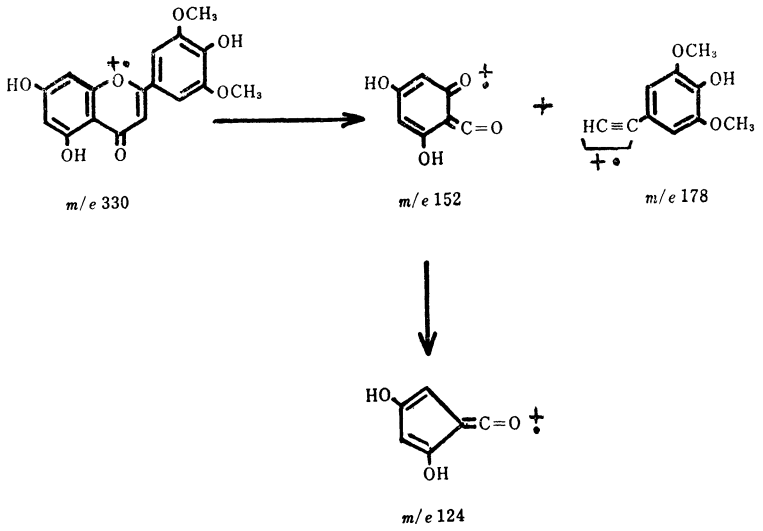
The grass was treated by the procedure to be described in the Experimental section. Consequently, luteolin (**3**) and luteolin-7-glucoside (**4**) were isolated. Moreover, the presence of arthraxin (**1**) was proved by obtaining arthraxin penta methyl ether from the methylated extract of the grass. These compounds were identified by mixed-melting-point determinations, by co-chromatography, and by a comparison of their UV and IR spectra with those of the authentic samples obtained from *Arthraxon hispidus Makino*.

Miscanthus sinensis Anderss

A boiling-water extract of the fresh leaves and stems (approximately 30 kg) of this plant was treated by a method similar to that previously described for the isolation of arthraxin from *Arthraxon hispidus Makino*.[1] The yellow crystalline needles (25 mg) thus obtained were presumed to be tricin (**5**) by studying their behavior.

*Tricin (**5**).* This compound had the empirical formula of $C_{17}H_{14}O_7$, and gave a positive color reaction of a hydroxyl flavone with several reagents. Its UV spectrum in ethanol showed peaks at 270 mμ (Band II) and at 350 mμ (Band I). The single well-defined peak of the II band indicates that the compound is a flavone which has one or three substituents in the B ring.[8] The bathochromic shift of 45 mμ of the I band on the addition of aluminum chloride indicates the presence of the 5-hydroxyl group. The fact that the addition of sodium ethylate induced a bathochromic shift of 65 mμ of the I band without decreasing its intensity indicates the presence of the 4'-hydroxyl group and the absence of the 3-hydroxyl group. The fact that the λ_{max} of the I band did not shift significantly on the addition of boric acid and sodium acetate shows the absence of *o*-hydroxyl groups. The NMR spectrum of the compound in deuterated DMSO indicated the presence of hydrogen atoms at the 3-(τ 3.09, s, 1H), 6-(τ3.82, d, 1H, J=2.5), 8-(τ 3.48, d, 1H, J=2.5), 2'-, and 6'-positions (2'- and 6'-equivalent aromatic protons; τ 2.74, s, 2H), the presence of methoxyl groups at the 3'- and 5'-positions (3'- and 5'-equivalent methoxyl groups; τ 6.12, s, 6H), and the presence of hydroxyl groups at the 5-(τ-2.90, 1H), 7-(τ-0.73, 1H) and 4'-(τ0.71, 1H) positions. The mass spectrum of the compound showed *m/e* 330 ($C_{17}H_{14}O_7$), 315 ($C_{16}H_{11}O_7$), 302 ($C_{16}H_{14}O_6$), 287 ($C_{15}H_{11}O_6$), 259 ($C_{14}H_{11}O_5$), 216 ($C_{12}H_8O_4$), 178 ($C_{10}H_{10}O_3$), 163($C_9H_7O_3$), 152($C_7H_4O_4$), 148 ($C_8H_4O_3$), and 124 ($C_6H_4O_3$) ions. The

6) T. Nakaoki and N. Morita, *J. Pharm. Soc. Japan*, **77**, 108 (1957).
7) Ref. 4, p. 324.
8) T. A. Geissman, J. B. Harborne, and M. K. Seikel, *J. Amer. Chem. Soc.*, **78**, 825 (1956).



Scheme

parent molecular ion is found at *m/e* 330 as the base peak. The *m/e* 178, 152, and 124 ions are derived from the fission in the heterocyclic ring as is shown in the scheme. These kinds of fragment ions are always observed in the mass spectra of the common flavonoid compound.

Only about 25 mg of this compound were isolated; the lack of a larger sample precluded extensive structural studies. However, Formula (**5**), which is identical with that of tricin, was deduced to be the most probable structure for the compound on the basis of the presented above data.

Phragmites communis Trinius

Fresh leaves and stems of this plant (approximately 20 kg) were treated much as *Miscanthus sinensis Anderss* has been. The isolated substance (*ca.* 10 mg) was identified with tricin by a mixed-melting-point determination, by co-chromatography, and by a comparison of its UV spectra with those of the authentic sample obtained from *Miscanthus sinensis Anderss.*

Experimental

All the melting points were uncorrected. The NMR spectra were measured at 60 MHz with a Hitachi R-20B H. R. NMR spectrometer. The chemical shifts were measured with tetramethylsilane as the internal standard. The high-resolution mass spectrum was determined by direct sample introduction using Hitachi RMS-4 and RMU-7 mass spectrometers.

Arthraxon hispidus Makino. Extraction and Isolation: Air-dried leaves and stems (approximately 70 kg) of the grass were treated as has been described in a previous arthraxin paper[1]; 2.85 g of arthraxin (**1**) were thus obtained. The ether solution (see Ref. 1) left after the arthraxin had been removed was concentrated to about one-third the volume and kept in a refrigerator. The deposited solid was recrystallized from acetone-benzene to give 1.95 g of aconitic acid (**2**) as colorless needles. The mother liquor of arthraxin and aconitic acid was concentrated almost to dryness. The residual substance was dissolved in 800 m*l* of boiling water and extracted with ether. The ether solution was then extracted three times with 300-m*l* portions of a saturated aqueous solution of sodium hydrogen carbonate and eight times with 300-m*l* portions of a 1% aqueous sodium carbonate solution. All the extracts were acidified with hydrochloric acid. The product from the hydrogen carbonate extract was recrystallized from alcohol-water to give 120 mg of luteolin-7-glucoside (**4**) as pale yellow micro-needles. The product from the carbonate extract was recrystallized from alcohol-water to give 260 mg of luteolin (**3**) as pale yellow needles.

Aconitic Acid (2): Recrystallization from acetone-benzene gave colorless needles; mp 190°C; λ_{max} (EtOH) 215 mμ; ν_{max} (KBr) 3030, 1730, 1690, 1420, 1285, 1220, 900, 850, and 740 cm^{-1}. The above data agreed with those obtained from authentic aconitic acid. Found: C, 41.37; H, 3.47. Calcd for $C_6H_6O_6$: C, 41.39; H, 3.47%.

Luteolin (3): Recrystallization from alcohol-water gave pale yellow micro-needles; mp 325°C dec., λ_{max} (EtOH) 255, 268 (inflection), 349 mμ, λ_{max} (EtOH+NaOAc) 269, 356 mμ; λ_{max} (EtOH+NaOAc+H_3BO_3) 260, 372 mμ; λ_{max} (EtOH+$AlCl_3$) 263, 277, 362, and 388 mμ; λ_{max} (EtOH+NaOEt) 275, 405 mμ. R_f value on Toyoroshi #51: 0.78 in *n*-butanol - acetic acid - water (4-1-2 by volume). The above results were the same as those reported for luteolin.[2-5] Found: C, 63.15; H, 3.61. Calcd for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52%.

Luteolin-7-glucoside (4): Recrystallization from alcohol-water gave yellow needles; mp 255°C; λ_{max} (EtOH) 257, 268 (inflection), 353 mμ; λ_{max} (EtOH+NaOH) 273, 405 mμ; λ_{max} (EtOH+NaOAc) 261, 362 and 410 mμ; R_f value on Toyoroshi #51: 0.51 in *n*-butanol - acetic acid - water (4-1-5 by volume). The data agreed with the data reported for luteolin-7-glucoside.[5-7] Found: C, 56.43; H, 4.92. Calcd for $C_{21}H_{20}O_{11}$: C, 56.25; H, 4.50%.

Hydrolysis of the Glucoside: Compound (**4**) was hydrolyzed by refluxing with 25% sulfuric acid for 11 hr. The aglycone was collected by filtration and recrystallized from ethanol-water to give luteolin, which was identified by its melting point (325°C), R_f values, and UV spectra, as has been described above. The filtrate was neutrilized with barium carbonate and then concentrated; it was used for the determination of the sugar by paper chromatography, which was carried out on Toyoroshi #51 in four solvent systems: R_f 0.18 in *n*-butanol - acetic acid - water (4-1-5 by volume),

0.27 in *n*- butanol - ethanol - water (4-1-5 by volume), 0.39 in coridine saturated with water, and 0.41 in phenol saturated with water. Identical values were obtained with an authentic sample of glucose.

Misanthus tinctorius Hackel. Extraction and Isolation: The grass was collected in Shiga Prefecture in September, 1970. One kg of air-dried leaves and stems of the grass were treated by the procedure previously described for the isolation of arthraxin from *Arthraxon hispidus Makino*. The resulting product was recrystallized from ethanol-water to give 6 mg of luteolin (**3**). The original mother liquor, which was left after extraction with ether to isolate luteolin, was extracted with 300 m*l* of ethyl acetate. The extract was concentrated under reduced pressure to yield a brownish-orange solid, which was then dissolved in 50 m*l* of boiling water. The solution was then saturated with 50 m*l* of ethyl acetate and kept for a month in a refrigerator. A yellowish crystalline substance which separated at the boundary of water and ethyl acetate was recrystallized from ethanol-water to give 8 mg of luteolin-7-glucoside (**4**) as yellow needles. In this way, approximately 10 kg of the air-dried grasses were processed, yielding *ca.* 50 mg of luteolin and *ca.* 65 mg of luteolin-7-glucoside.

The remaining solution, from which luteolin was obtained, was evaporated to dryness. The residue was dissolved in 200 m*l* of dry acetone and was heated under reflux with 15 m*l* of dimethyl sulfate and 20 mg of anhydrous potassium carbonate for 56 hr, until the ferric chloride reaction became negative. After the inorganic salt has been removed by filtration, the filtrate was evaporated under reduced pressure. The residue was dissolved in a small portion of a mixture of acetone and benzene (1 : 4) and passed through a silica-gel (Kieselgel less than 0.08 mm, Merck) column. Elution was carried out successively with the following solvents: 500 m*l* of benzene, 1000 m*l* of benzene-ethyl acetate (1 : 1), 1000 m*l* of ethyl acetate, and 1000 m*l* of ethyl acetate-acetone (1 : 1). The fraction which was eluted with ethyl acetate-acetone (1 : 1) was evaporated, and the residue was recrystallized from ethanol to give *ca.* 120 mg of arthraxin penta methyl ether as colorless needles.

*Luteolin (**3**) and Luteolin-7-glucoside (**4**)*: The identifications of these compounds were based on the agreement of the melting points, R_f values, and UV spectra with those of the luteolin[2-5] and luteolin-7-glucoside[5-7] isolated from *Arthraxon hispidus Makino*.

Arthraxin Penta Methyl Ether: Recrystallization from ethanol gave colorless needles; mp 204°C; λ_{max} (EtOH) 247, 266, and 338 mμ; ν_{max} (KBr) 2905, 1635, 1590, 1510, 1410, 1395, 1315, 1255, 1200, 1120, 1040, and 835 cm^{-1}. This substance was identified with arthraxin penta methyl ether by a mixed-melting-point determination and by co-chromatography on tlc with authentic sample[1] obtained from *Arthraxon hispidus Makino*.

*Miscanthus sinensis Anderss and Phragmites communis Trinius. Tricin (**5**)*: Recrystallization from ethanol-water gave yellow needles; mp 282°C; Mass (high-resolution mass spectrum): M 330.0730, Calcd for $C_{17}H_{14}O_7$, M 330.0739; λ_{max} (EtOH) 245 (inflection), 270, 350 mμ; λ_{max} (EtOH+NaOAc+H_3BO_3) 272, 355 mμ; λ_{max} (EtOH+$AlCl_3$) 280, 305, 370, and 395 mμ; λ_{max} (EtOH+NaOEt) 262, 325, and 415 mμ. These data are nearly equal to those reported for tricin.[9-11]

The authors wish to express their thanks to Mr. Kyuzaburo Furuhashi, Tokyo Kyoiku University, for the microanalyses and the NMR measurements; to Mr. Hidetora Okuyama for sending a sample of *Arthraxon hispidus Makino* collected on Hachijo Island; to Mr. Tomohiko Takahashi for sending some grass of *Miscanthus tinctorius Hackel* collected in Shiga Prefecture, and to Dr. Tsuguo Tateoka, National Science Museum, for giving an expert opinion on the grass of *Miscanthus tinctorius Hackel*.

9) Ref. 4, p. 114.
10) J. A. Anderson and A. G. Perkin, *J. Chem. Soc.*, **1931**, 2624.
11) W. S. Ferguson, DE B. Ashworth, and R. A. Terry, *Nature* **163**, 606 (1949).
12) J. B. Harborne, *Chem. Ind.*, (London) **1958**, 1950.