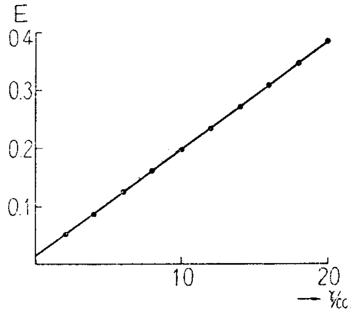

Fig. 5.

Standard Curve

The author gratefully acknowledges the guidance and advice of Assist.-Prof. Bonro Kobayashi. Thanks are also due to Prof. Takio Iwamoto for his encouragements.

Summary

An assay method suitable for reducing sugars present in 0.02 cc. of normal blood was described.

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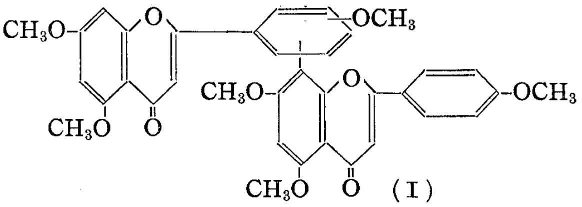
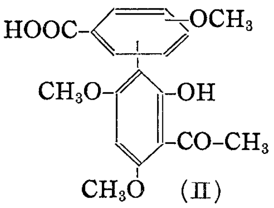
125. **Nobusuke Kawano** : Studies on the Structure of Sciadopitysin, a Flavonoid from the Leaves of *Sciadopitys verticillata* Sieb. et Zucc. VII.[*1]

(*Pharmaceutical Faculty, University of Nagasaki*[*2])

In the preceding paper,[1] it was deduced that sciadopitysin trimethyl ether, $C_{36}H_{30}O_{10}$, consists of xanthoxylin, substance B (a phenolic acid, $C_{18}H_{18}O_7$), and anisic acid, and that its structure must be represented by a tentative formula (I) because it gave these three fragments in considerably high yield when treated with methanolic barium hydroxide solution. Consequently, substance B must have a biphenyl structure (II) instead of a desoxybenzoin derivative as suggested earlier.[2]


(I)


(II)

On oxidation with alkaline potassium permanganate solution, substance B monomethyl ether produced a monoketodicarboxylic acid (III), $C_{19}H_{18}O_9$, m.p. 216~217°(decomp.), which gave *p*-nitrophenylhydrazone of m.p. 223~224°(decomp.) and 2,4-dinitrophenylhydrazone of m.p. 230~231°(decomp.). When kept at 215~220°, this acid (III) decomposed rapidly to give a monocarboxylic acid (IV), $C_{17}H_{18}O_6$, m.p. 231°, which was subsequently

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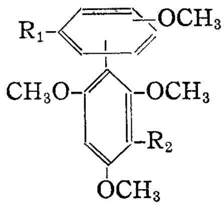
[*1] This paper constitutes Part XXXVI of "Chemical Consituentes of the Plants of Coniferae and Allied Oders" by T. Kariyone. Part XXXV : Ref. 1).

[*2] Showa-machi, Nagasaki (河野信助).

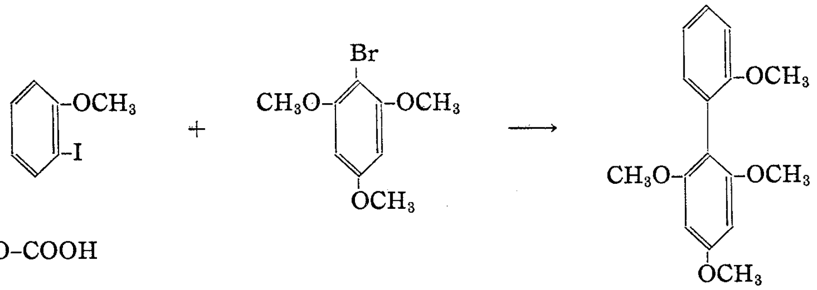
1) Part VI : Yakugaku Zasshi, **79**, 1182(1959).

2) N. Kawano : *Ibid.*, **76**, 457(1956) [C.A., **50**, 16759(1956)].

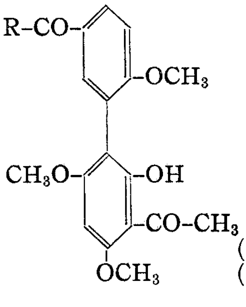
decarboxylated into a neutral compound (V), $C_{16}H_{18}O_4$, m.p. 111°, in a boiling quinoline solution with copper powder. As the most likely alternative structure for (V), 2,2′,4,6- and 2,4,4′,6-tetramethoxybiphenyl were newly synthesized by the Ullmann reaction from 2- or 4-iodoanisole and bromophloroglucinol trimethyl ether, and these compounds melted at 110~111° and 102~103°, respectively.



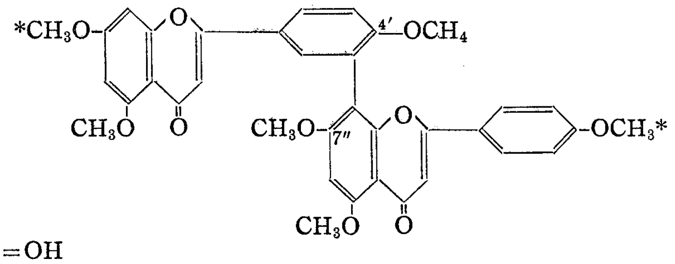
(III) R_1=COOH, R_2=CO-COOH
(IV) R_1=COOH, R_2=H
(V) R_1=R_2=H



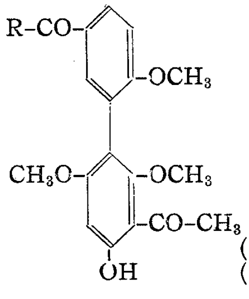
The compound (V) was identified with a synthetic 2,2′,4,6-tetramethoxybiphenyl through admixture and ultraviolet absorption spectra. The substance B was oxidized in alkaline potassium permanganate solution into 4-methoxyisophthalic acid which was recognized by admixture. These two experimental results suggest the complete formula (VI) for the structure of substance B, instead of the foregoing tentative formula (II). Therefore, sciadopitysin trimethyl ether can be decidedly represented by the structural formula (VII). Substance A,[2] a phenolic diketone, $C_{19}H_{20}O_6$, must then have the structure (VIII) because it was also oxidized into 4-methoxyisophthalic acid like substance B. It goes without saying that the chemical behaviors of substances A and B formerly reported[2,3] are entirely consistent with formula (VIII) and (VI), respectively. This includes the suggestion that the formulae (VI), (VII), and (VIII) are more preferable than (VI′), (VII′), and (VIII′) from the consideration of experimental observations that the phenolic hydroxyl group of substance A is not easily methylated, as mentioned earlier,[2] though the final decision depends on further studies.



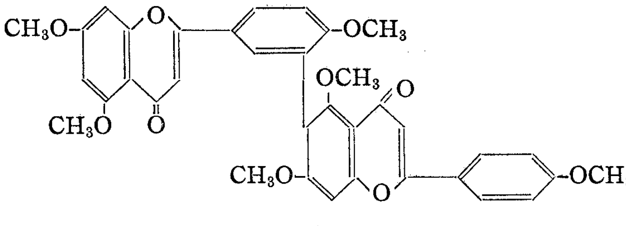
(VI) R=OH
(VIII) R=CH_3



(VII)

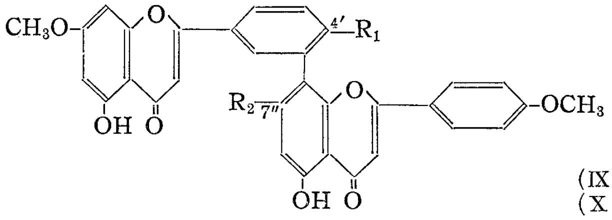


(VI′) R=OH
(VIII′) R=CH_3



(VII′)

It is obvious that the asterisked two methoxyl groups in the formula (VII) were present in sciadopitysin before methylation because sciadopitysin afforded anisic acid, *p*-methoxyacetophenone, and 2,6-dihydroxy-4-methoxyacetophenone besides an oxoflavone and a carboxyflavone[*3] by degradation in a boiling aqueous potassium hydroxide solution.[4] Furthermore, as reported previously,[5] sciadopitysin has two hydroxyl groups which are hard to methylate and they must be in 5-positions of the two flavone rings of sciadopitysin. The other two methoxyl groups (4′ and 7″) in the formula (VII) must be a hydroxyl and a methoxyl group in sciadopitysin because it has three hydroxyl groups.[5] To sum up these findings, the structure of sciadopitysin can be clearly represented by the alternative formula (IX) or (X) which is a new-type bisflavonoid that have never been reported.



(IX) $R_1=OCH_3$, $R_2=OH$
(X) $R_1=OH$, $R_2=OCH_3$

In order to elucidate this alternative, degradation of sciadopitysin triethyl ether is being carried out expecting to obtain either 4-ethoxy- or 4-methoxyisophthalic acid.[*4]

Grateful acknowledgment is offered to Dr. T. Kariyone for his interest and encouragement throughout this work. Thanks are also due to Mr. H. Miura and Miss H. Yonaha for their technical assistance in preparing some of the materials used in this work, and to Miss H. Ohta for elemental analytical data. The author also acknowledges a Grant-in-Aid for Scientific Research from the Ministry of Education.

Experimental[*5]

Monoketodicarboxylic Acid (III)—Substance B monomethyl ether ($C_{19}H_{20}O_7$, m.p. 206~207°(decomp.), 500 mg.) was dissolved in 1% KOH solution (50 cc.) and warmed with 2% $KMnO_4$ solution (25 cc.) on a steam bath for 3 hr. After being decolorized with Na_2SO_3 and separated from MnO_2, the filtrate was combined with washings, concentrated to about 30 cc., acidified with 10% HCl, and allowed to stand overnight. White precipitate was collected and recrystallized once from AcOEt to faint yellow needles (480 mg.), m.p. 210~212°(decomp.). Further recrystallizations gave almost colorless needles, m.p. 216~217°(decomp.). *Anal.* Calcd. for $C_{19}H_{18}O_9$: C, 58.46; H, 4.65; mol. wt., 390.3. Found : C, 58.34; H, 4.76; mol. wt., 375.2. λ_{max}^{EtOH} 247 mμ (ε 29,600).

p-Nitrophenylhydrazone : Yellow minute prisms (from 50% AcOH), m.p. 223~324°(decomp.). *Anal.* Calcd. for $C_{25}H_{23}O_{10}N \cdot H_2O$: C, 55.25; H, 4.64; N, 7.73. Found : C, 55,62; H, 4.43; N, 7.20.

2,4-Dinitrophenylhydrazone : Orange-yellow minute prisms (from 80% EtOH), m.p. 230~231°(decomp.). *Anal.* Calcd. for $C_{25}H_{22}O_{12}N_4 \cdot H_2O$: C, 51.02; H, 4.11; N, 9.52. Found : C, 50.69; H, 4.24; N, 9.06.

Monocarboxylic Acid (IV)—The foregoing monoketodicarboxylic acid (III), m.p. 210~212° (300 mg.), dispersed on the flat bottom of a 30-cc. Erlenmeyer flask was heated at 215~220° in an oil bath. A rapid evolution of gas occurred and after about 5 min., the reaction ended. A brownish hard syrup thus obtained was induced to crystallize by addition of EtOH. The filtered crystals (205 mg. in air-dried state) were recrystallized twice from 50% *iso*-PrOH to colorless prisms (70 mg.), m.p. 231°. *Anal.* Calcd. for $C_{17}H_{18}O_6$: C, 64.14; H, 5.70; mol. wt., 318.3. Found : C, 64.22; H, 5.78; mol. wt., 323.8. λ_{max}^{EtOH} 247 mμ (ε 20,400).

Tetramethoxybiphenyl (V)—A mixture of the above-mentioned monocarboxylic acid (IV), m.p. 229~230°(100 mg.), Cu powder (300 mg.), and quinoline (3 cc.) was refluxed for 7 hr., cooled, and filtered. Cu dust was washed with Et_2O. The filtrate and washings were combined and added dropwise

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[*3] Its molecular formula previously reported, $C_{26}H_{20}O_9$, must be corrected to $C_{24}H_{18}O_8$ in accordance with new results obtained recently.

[*4] A personal communication from Prof. W. Baker (University of Bristol) said that he had been investigating ginkgetin and sciadopitysin, and had reached a conclusion similar to that described in this paper by entirely independent work.

[*5] All m.p.s are uncorrected.

3) T. Kariyone, N. Kawano, M. Maeyama : Yakugaku Zasshi, **79**, 382(1959).

4) T. Kariyone, N. Kawano : *Ibid.*, **76**, 451(1956).

5) *Idem.* : *Ibid.*, **76**, 448(1956).

into a mixture of 35% HCl (4 cc.) and water (6 cc.) under cooling and stirring. The resultant liquor was extracted thoroughly with Et_2O. The combined Et_2O layer was washed twice with 5% H_2SO_4, dried, and evaporated, leaving a brownish viscous oil (about 100 mg.). The oil was dissolved in EtOH (2 cc.), allowed to stand, and crystals formed were collected. The crystals (m.p. 106~110°, 60 mg.) were dissolved in benzene, purified by chromatography through a column of alumina (3 g.), and recrystallized from EtOH to colorless plates (25 mg.), m.p. 111°. *Anal.* Calcd. for $C_{16}H_{18}O_4$: C, 70.05; H, 6.61. Found : C, 69.87; H, 6.43. λ_{max}^{EtOH} 247 mμ (ε 8,850). It was identical with synthesized 2,2′,4,6-tetramethoxybiphenyl by admixture.

Oxidation of Substance B—To a stirred solution of substance B (170 mg.) in 3% NaOH solution (10 cc.), 2% $KMnO_4$ (40 cc.) was added dropwise during 1 hr. at 40~50°. After the solution was decolorized, MnO_2 was filtered off and washed. The combined filtrate was concentrated to 20~25 cc., acidified with 20% HCl, and extracted 3 times with Et_2O. Ethereal layers were combined, dried, and evaporated, leaving an oily residue which gave some crystals (about 35 mg.) by addition of a few drops of water. They were filtered and recrystallized twice from AcOEt, m.p. 264~265°. *Anal.* Calcd. for $C_9H_8O_5$ (4-Methoxyisophthalic acid) : C, 55.10; H, 4.11. Found : C, 54.84; H, 4.18. Mixed m.p. with a synthetic 4-methoxyisophthalic acid[6] (m.p. 264°), 264~265.°

The dimethyl ester was prepared with diazomethane as usual. Colorless needles (from petr. ether), m.p. 94~95°. *Anal.* Calcd. for $C_{11}H_{12}O_5$ (Dimethyl 4-methoxyisophthalate) : C, 58.92; H, 5.40. Found : C, 59.05; H, 5.59. It was identical with an authentic sample by admixture.

Oxidation of Substance A—A mixture of substance A (150 mg.), acetone (5 cc.), and 5% NaOH (3 cc.) was refluxed on a steam bath to give a clear solution, water (10 cc.) was added, and acetone was evaporated. This solution was warmed with 2% $KMnO_4$ (60 cc.) by the same procedure as for substance B and 4-methoxyisophthalic acid was obtained (10 mg.), m.p. and mixed m.p. 264~265°.

2,2′,4,6-Tetramethoxybiphenyl (Ullmann Reaction)—To a stirred mixture of 2-iodoanisole (2.34 g.) and bromophloroglucinol trimethyl ether (0.83 g.), Cu powder (1.5 g.) was added during 20 min. in an oil bath at 225~230°. After further 2 hr.'s heating the mixture was extracted with $CHCl_3$ (40 cc.), dried, and evaporated to give a crystalline residue (1.6 g.). A little volume of EtOH was added to the residue, stirred, and filtered to separate crude crystals of m.p. 146~154°(460 mg.), which melted at 155~156°(reported[7] m.p. 154~155°) after recrystallizations from EtOH. *Anal.* Calcd. for $C_{14}H_{14}O_2$ (2,2′-Dimethoxybiphenyl) : C, 78.48; H, 6.59. Found : C, 78.20; H, 6.57.

The EtOH mother liquor was evaporated to dryness, the residue was dissolved in benzene (3 cc.), and purified by chromatography on a column of alumina (10 g.), eluting with benzene. Intermediate fractions left an oil, which was collected (720 mg.) and purified once more by chromatography as above. The crude crystals gathered from the intermediate fractions were recrystallized three times from EtOH to colorless plates (25 mg.), m.p. and mixed m.p. with (V), 110~111°. *Anal.* Calcd. for $C_{16}H_{18}O_4$ (2,2′,4,6-Tetramethoxybiphenyl) : C, 70.05; H, 6.61. Found : C, 69.89; H, 6.87. λ_{max}^{EtOH} 247 mμ (ε 8,800).

Later fractions gave colorless needles (200 mg.), m.p. 153~154°, from EtOH. *Anal.* Calcd. for $C_{18}H_{22}O_6$ (2,2′,4,4′,6,6′-Hexamethoxybiphenyl[8]) : C, 64.65; H, 6.63. Found : C, 64.26; H, 6.80.

2,4,4′,6-Tetramethoxybiphenyl—By the same procedure as described above, 4-iodoanisole (2.34 g.) and bromophloroglucinol trimethyl ether (0.83 g.) gave following three kinds of crystals through chromatographic separation and recrystallization from EtOH. (i) Colorless scales (250 mg.), m.p. 177~178° (4,4′-dimethoxybiphenyl[9]). (ii) Colorless prisms (150 mg.), m.p. 102~103°. *Anal.* Calcd. for $C_{16}H_{18}O_4$ (2,4,4′,6-Tetramethoxybiphenyl) : C, 70.05; H, 6.61. Found : C, 69.91; H, 6.79. Admixture with (V) gave m.p. 85~95°. (iii) Colorless needles (35 mg.), m.p. and mixed m.p. with the foregoing hexamethoxybiphenyl, 153~154°.

Summary

1) 2,2′,4,6-Tetramethoxybiphenyl was obtained from substance B monomethyl ether, $C_{19}H_{20}O_7$, by oxidation followed by decarboxylation, (II)→(III)→(IV)→(V), and it was also newly synthesized by the Ullmann reaction.

2) 4-Methoxyisophthalic acid was obtained from substance A (VIII) and B (VI) by oxidation.

3) The structure of sciadopitysin trimethyl ether was established as a new-type bisflavonoid of formula (VII) or (VII′), the former being preferable.

4) Formula (IX) or (X) was deduced as the structure of sciadopitysin.

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6) F. D. Chattaway, F. Calvet : J. Chem. Soc., **1928**, 2913.
7) K. Lederer : Ber., **53**, 713(1920).
8) W. Riedl : Ann., **597**, 151(1955).
9) A. Gillmeister : Ber., **30**, 2849(1897).