UDC 547.972.2/.3 : 582.952.2

13. **Naokata Morita** : Studies on the Flavonoid of Sesame Leaves. I. On the Structure of the Glycoside, Pedaliin.

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Sesame is extensively cultivated in the world and bears seeds of black, white, or pale yellow in color. The seed contains approximately 45~55% of fatty oil which is also used extensively for medicinals, food, and cosmetics. In the course of studies on the flavonoid in the plant world, a new flavonoid was extracted from the leaves of sesame (*Sesamum indicum* L.) (Pedaliaceae) and the structure of this flavonoid is described in this paper.

A dried leaves of sesame were extracted with methanol and a product was obtained as pale yellow microneedles, m.p. 254°. This substance colored greenish brown to ferric chloride solution, was positive to the flavone reaction, and its dilute acid hydrolyzate reduced the Fehling solution. Its analytical values suggested the molecular formula of $C_{22}H_{22}O_{12}\cdot 2\frac{1}{2}H_2O$.

Hydrolysis of this substance with 10% sulfuric acid afforded an aglycone in 65.92% yield, which suggests that a sugar bonded to it is one mole. The aglycone formed yellow minute needles, m.p. 300~301°; coloring brown to ferric chloride and giving positive flavone reaction. Its analytical values gave the molecular formula of $C_{15}H_9O_6$-(OCH_3).

The sugar portion was treated by the usual process and glucose was detected by paper partition chromatography. Its osazone of yellow needles, m.p. 207°, showed no melting point depression on admixture with glucosazone, m.p. 206~207°. With the sugar determined, the hydrolysis of this flavonoid may be written as follows :

$$C_{22}H_{22}O_{12} + H_2O = C_{16}H_{12}O_7 + C_6H_{12}O_6$$

Acetylation of the aglycone afforded colorless needles, m.p. 242~243°, while its methylation with diazomethane gave pale yellow needles, m.p. 189~190°. This methylated substance colors purplish brown to ferric chloride, possesses one non-methylated hydroxyl, which was assumed to be the chelated hydroxyl in 5-position. The product possesses four methoxyl groups and its molecular formula agrees with $C_{15}H_6O_3(OCH_3)_4$. Further methylation with dimethyl sulfate gave colorless prismatic crystals, m.p. 172~173°, which failed to color with ferric chloride. This substance has five methoxyls and agrees with the formula $C_{15}H_5O_2(OCH_3)_5$. It follows, therefore, that the aglycone possesses one methoxyl and four hydroxyls. Such a substance has not been mentioned in any literature and the aglycone is named pedalitin, and the glycoside, pedaliin.

Methylation of pedaliin with diazomethane effects complete methylation. Hydrolysis of the methylated product with 5% sulfuric acid and recrystallization of the aglycone so formed from 50% methanol afforded pale yellow needles, m.p. 221~222°, which remained colorless to ferric chloride. This fact is analogous to the case of luteolin 5,3′,4′-trimethyl ether, which does not color to ferric chloride but gives marked purplish brown coloration when the hydroxyl in the 5-position is free. The methylated aglycone possesses four methoxyls and agrees with the molecular formula of $C_{15}H_6O_3(OCH_3)_4$. Further methylation of this product with diazomethane gives a completely methylated product as colorless prisms, m.p. 172~173°, undepressed on admixture with the completely methylated product of m.p. 172~173° described above.

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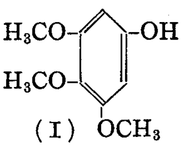
Demethylation of pedalitin with hydriodic acid gave a product as yellow microneedles, m.p. over 325°, coloring dark brown to ferric chloride and corresponding to the formula $C_{15}H_{10}O_7 \cdot 2H_2O$. Acetylation of the demethylated product gave colorless needles, m.p. 245～246°, which showed melting point depression on admixture with pedalitin acetate of m.p. 242～243°, melting at 220°, indicating that demethylation had been effected.

In order to determine the position of hydroxyl groups, alkali fusion of pedalitin was carried out and protocatechuic acid, m.p. 197～198°, was obtained as a phenol-carboxylic acid but phenol was not obtained. This suggests that the phenol is a polymerizable, polyhydroxyphenol.

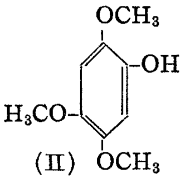
Decomposition of pedalitin with 30% alkali afforded a product from phenolic portion as colorless needles, m.p. 116～117°; giving negative pine-splinter reaction, coloring bright green to ferric chloride, and rose red on addition of sodium hydrogen carbonate. Considering the formation of protocatechuic acid by alkali fusion, this product was assumed to be 3,4-dihydroxyacetophenone and the fact was confirmed by admixture with synthesized 3,4-dihydroxyacetophenone, m.p. 117°.

The mother liquor obtained after removal of this acetophenone failed to afford polyhydroxyphenol and the acid portion also did not provide any substance. This indicates the phenyl group in the flavone does not possess any methoxyl but has two hydroxyls which are in 3- and 4-positions. The formation of a ketone compound indicates that the glycoside is a flavone. Decomposition of pedalitin with barium hydroxide in nitrogen atmosphere also failed to give any phenol.

In order to determine the phenolic substances, complete methylation of pedalitin was carried out and the product therefrom was decomposed with 25～30% alkali. The phenolic portion afforded colorless prismatic needles, m.p. 140～141°, which colored dark brown to ferric chloride, and possessed three methoxyls, the analytical values agreeing with the molecular formula of $C_6H_3O(OCH_3)_3$. Four possible formulae (I to IV) may be considered for this trimethoxyphenol obtained by alkaline decomposition of the flavone.



(I)



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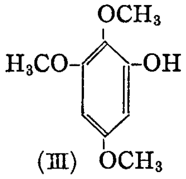
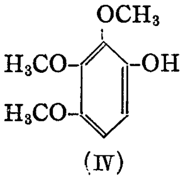


(III)



(IV)

The incomplete methylation of pedalitin by diazomethane indicates the presence of chelated hydroxyl in 5-position and therefore, formula (IV) would be impossible. This leaves (I), (II), and (III) as the possible structure.

Pectolinarigenin[1] is 5,7-dihydroxy-6,4′-dimethoxyflavone and wogonin[2] is 5,7-dihydroxy-8-methoxyflavone. Alkaline hydrolysis of their completely methylated product should produce trimethoxyphenols corresponding to formula (I) from pectolinarigenin and to formula (III) from wogonin. In order to obtain (I) and (III) and compare them with the phenol in question, alkaline decomposition with 25～30% alkali was carried out on completely methylated products of pectolinarigenin and wogonin extracted from natural source, and (I) was obtained as colorless plates, m.p. 142～143°, and (II) as colorless prisms, m.p. 112～114°. The phenol obtained by alkaline decomposition of the flavone was admixed with (I) and (III), and the substance was identified with (I). Therefore, the three hydroxyls in the benzene ring of the chromone are in 5-, 6-, and 7-positions, and

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[1] T. Nakaoki, N. Morita : Yakugaku Zasshi, **75**, 172(1955).

[2] K. Shibata, S. Iwata, M. Nakamura : Acta Phytochim., **1**, 132(1923).

the methoxyl must be among these. Since the hydroxyl in 5-position seems to be chelated, the methoxyl must be in 6- or 7-position.

The sugar must be bonded to the flavone glycoside in 7-position and this leaves 6-position as the possible one for the methoxyl in pedalitin. In order to confirm this point, alkaline decomposition was carried out on the methylated product of pale yellow needles, m.p. 189~190°, obtained from pedalitin with diazomethane, and the hydrolyzed product of pale yellow needles, m.p. 221~222°, obtained by complete methylation of the glycoside, pedaliin, with diazomethane, followed by hydrolysis. A phenolic substance of m.p. 76~77° was obtained from the former substance but not from the latter. This is illustrated in Chart 1.

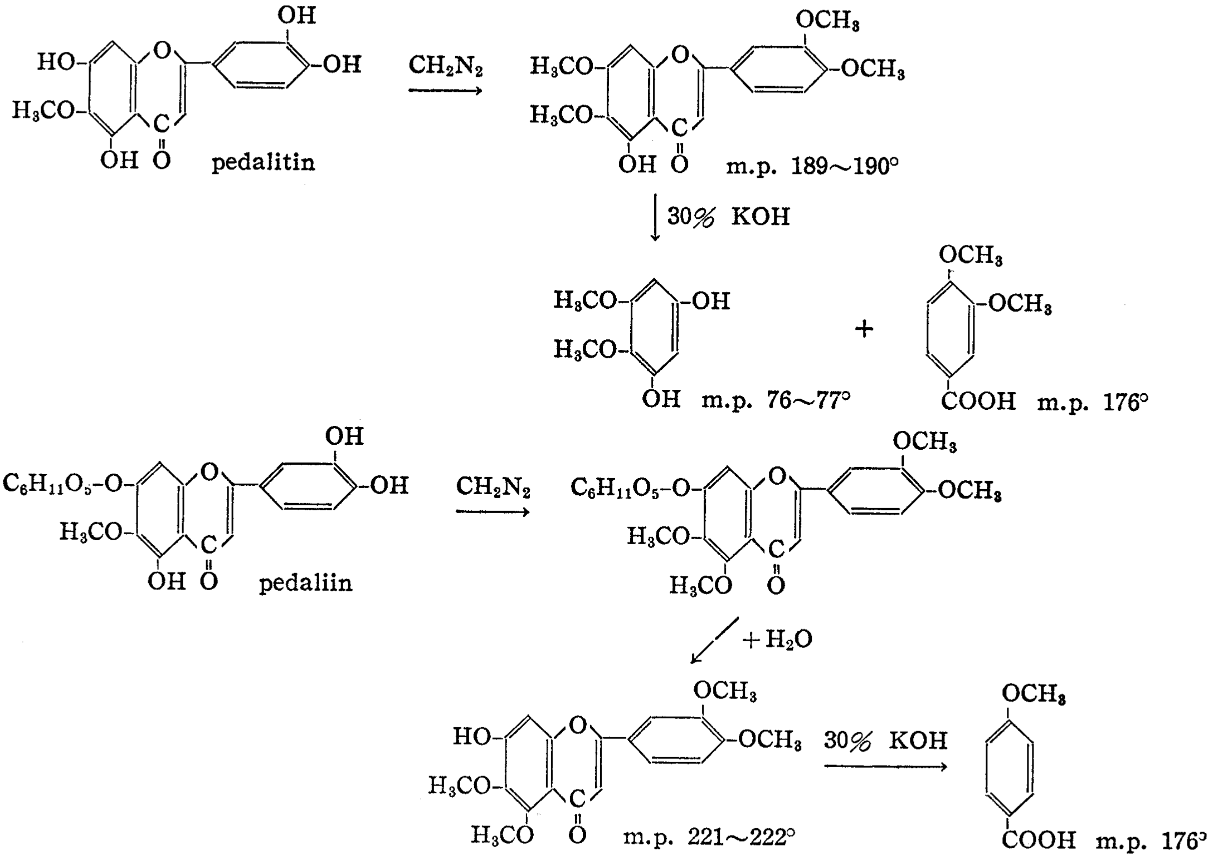

Chart 1.

A phenolic substance of colorless needles, m.p. 76~77°, was obtained by alkaline decomposition of the substance considered to possess chelated hydroxyl in 5-position and this phenolic substance colored reddish brown to ferric chloride and violet to pine-splinter reaction, the properties agreeing entirely with those of 4,5-dimethoxyresorcinol. The fact was confirmed by admixture with the synthesized product of m.p. 76°, and this proved that the hydroxyl in pedalitin not methylated by diazomethane is in its 5-position and that the methoxyl is in 6- or 7-position.

The acid substance obtained as colorless needles, m.p. 176°, was identified by admixture with veratric acid, m.p. 179°. The reason why 4,5-dimethoxyresorcinol was not obtained by alkaline decomposition of the substance forming pale yellow needles, m.p. 221~222°, is probably due to the difficulty of alkylation of the hydroxyl chelated to the carbonyl in the flavone as well as its easy liberation, and the substance is assumed to have been formed by polymerization of the phenol produced by demethylation of the methoxyl in 5-position.

Failing to obtain 4,5-dimethoxyresorcinol by alkaline decomposition of the substance assumed to be 5,6,3′,4′-tetramethoxy-7-hydroxyflavone, the methylated pedalitin with diazomethane, m.p. 189~190°, and pale yellow needles, m.p. 221~222°, obtained by complete methylation of pedaliin with diazomethane, followed by hydrolysis, were ethylated and the products were submitted to alkaline decomposition, in order to compare the phenol obtained. This is illustrated in Chart 2.

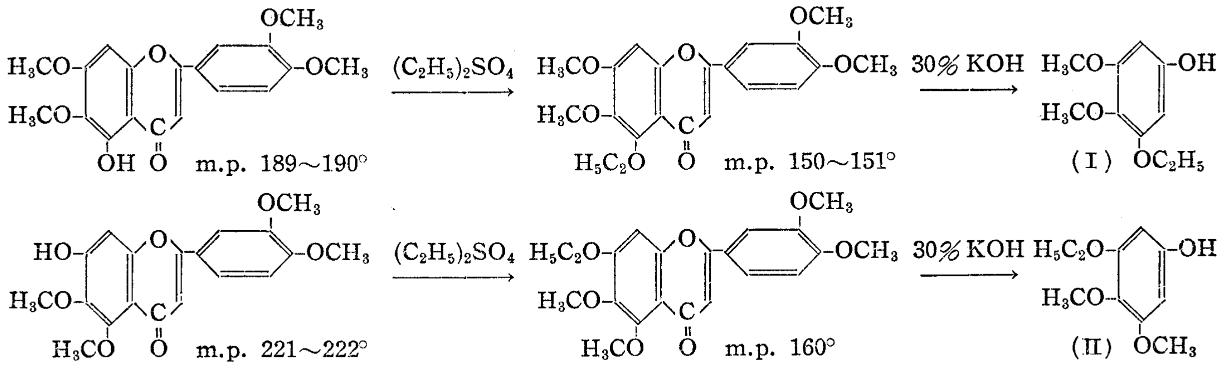

Chart 2.

These tetramethoxy-hydroxyflavones were ethylated with diethyl sulfate and the products were decomposed with 30% alkali, affording pale yellow, viscous phenols, respectively. These phenols both colored pale purplish black to ferric chloride and were difficult to crystallize that they were compared by paper partition chromatography.

TABLE I. Rf Values on Paper Chromatogram

Solvent system	60% AcOH	15% AcOH	BuOH:AcOH:H_2O (4:1:5)
Phenol-I	0.945	0.65	0.93
Phenol-II	0.945	0.65	0.93

This result has shown that the two phenols are identical and, consequently, the one methoxyl in the pedalitin molecule must be in 6-position.

Further confirmation of the position of the methoxyl in pedalitin was attempted through comparison of its ultraviolet spectrum with those of luteolin 7,3′,4′-trimethyl ether and luteolin 5,3′,4′-trimethyl ether, which are considered to be analogous to

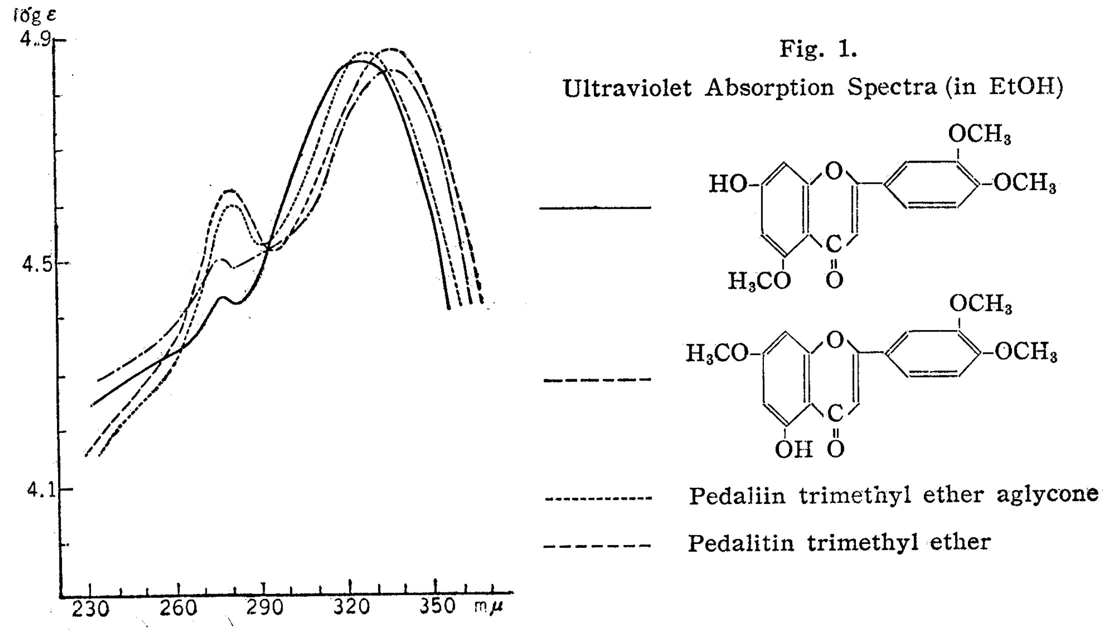

Fig. 1.
Ultraviolet Absorption Spectra (in EtOH)

pedalitin trimethyl ether having hydroxyls in 5- and 7-positions. The spectral curves of these compounds are shown in Fig. 1.

The absorption maximum of luteolin trimethyl ether possessing chelated hydroxyl in 5-position is at 340 mμ, while that possessing the hydroxyl in 7-position shows absorption maximum at 330 mμ. It was found that the aglycone of completely methylated pedaliin, i.e. substance considered to be 5,6,3′,4′-tetramethoxy-7-hydroxyflavone, and methylated product of pedalitin considered to have a hydroxyl in 5-position respectively showed absorption maximum at 330 and 340 mμ. Consequently, the aglycone of completely methylated pedaliin has a hydroxyl in 7-position and this is the bonding position of glucose, and the hydroxyl in pedalitin which is not methylated by diazomethane is the chelated hydroxyl in 5-position. This has proved that the methoxyl is in 6-position.

These experimental results has shown that the flavonoid obtained from the sesame leaves is 5,3′,4′-trihydroxy-6-methoxyflavone 7-glucoside.

Experimental

Extraction of the Glycoside, Pedaliin, and Its Properties—The dried leaves of sesame (*Sesamum indicum* L.) was digested twice with MeOH, the extract was evaporated, and the residue was boiled for a short time with MeOH. Crystals precipitated out on allowing the solution to stand. Yield, 0.3%. This was recrystallized from pyridine-water and formed pale yellowish white microneedles, m.p. 254°(decomp.). Mg+HCl—pale pink to orange brown. Zn+HCl—pale pink to colorless. Greenish brown to $FeCl_3$. $[\alpha]_D^{19}$ +27.2°(c=0.294, pyridine:EtOH=1:9). Rf 0.42 (BuOH:AcOH:H_2O=4:1:2), the spot appearing brown to ultraviolet light. This substance is insoluble in Et_2O, $CHCl_3$, and benzene, and sparingly soluble in MeOH and EtOH. The dilute acid hydrolyzate reduces the Fehling solution. *Anal.* Calcd. for $C_{22}H_{22}O_{12}\cdot2\frac{1}{2}H_2O$: C, 50.47; H, 5.16; 2.5 H_2O, 8.60. Found : C, 50.46; H, 6.015; H_2O, 8.75.

Hydrolysis of Pedaliin; Formation of Pedalitin—Pedaliin was warmed on a water bath for 2 hr. with 10% H_2SO_4, the precipitate formed was collected by filtration, and washed with water. The dried precipitate was crystallized from MeOH and formed yellow minute needles, m.p. 300~301°. Mg+HCl—orange-brown. Rf 0.51 (the solvent same as above), the spot appearing brown to ultraviolet light. The substance dissolves in conc. HCl and conc. H_2SO_4, forming a yellow solution, in 10% KOH to form orange red solution wnich turns brown, and in 10% Na_2CO_3 to form yellow solution, which turns greenish brown from the surface and changes to a tan-colored solution. *Anal.* Calcd. for $C_{16}H_{12}O_7\cdot\frac{1}{2}H_2O$: C, 59.07, H, 4.00; 1 CH_3O, 9.53; 0.5 H_2O, 2.76. Found : C, 58.81; H, 4.10; CH_3O, 10.04; H_2O, 2.57.

The mother liquor left after removal of pedalitin was reduced to a syrup by the usual process and submitted to paper partition chromatography. The spot appeared at Rf 0.36 (glucose, 0.36) when developed with 75% phenol and at Rf 0.16 (glucose, 0.16) when developed with BuOH:AcOH:H_2O mixture (4:1:5), the spots appearing yellowish brown on spraying 0.1*N* aniline hydrogen phthalate. The osazone formed yellow needles, m.p. 207°, undepressed on admixture with glucosazone, m.p. 206°.

Acetylation of pedalitin by the usual method and recrystallization from MeOH afforded colorless needles, m.p. 242~243°. *Anal.* Calcd. for $C_{24}H_{20}O_{11}\cdot\frac{1}{2}H_2O$: C, 58.41; H, 4.05; 0.5 H_2O, 1.82. Found: C, 58.25; H, 4.195; H_2O, 1.70.

Methylation of Pedaliin—An excess of Et_2O solution of CH_2N_2 was applied to pedaliin and the yellowish brown, syrupy substance thereby obtained, giving negative $FeCl_3$ reaction, was hydrolyzed with 5% H_2SO_4. The product recrystallized from 50% MeOH to pale yellow needles, m.p. 221~222°, remaining almost colorless to $FeCl_3$ test. *Anal.* Calcd. for $C_{19}H_{18}O_7$: C, 63.68; H, 5.03; 4 CH_3O, 34.66. Found : C, 63.37; H, 5.11; CH_3O, 34.18. UV λ_{max}^{EtOH} mμ (log ε) : 283 (4.59), 330 (4.86). (Luteolin 5,3′,4′-trimethyl ether : 278 (4.46), 330 (4.85)).

This product was further methylated with a large excess of Et_2O solution of CH_2N_2 and the product was recrystallized from MeOH, forming colorless prisms, m.p. 172~173°; negative to $FeCl_3$ color reaction. No depression in m.p. occurred on admixture with the product of m.p. 172~173°, obtained by complete methylation of pedalitin with Me_2SO_4.

Methylation of Pedalitin—i) With CH_2N_2 : Et_2O solution of CH_2N_2 was added to pedalitin suspended in a small quantity of MeOH and the product was recrystallized from 70% MeOH, forming pale yellow needles, m.p. 189~190°; coloring purplish brown to $FeCl_3$. *Anal.* Calcd. for $C_{19}H_{18}O_7$: C, 63.68; H, 5.03; 4 CH_3O, 34.66. Found : C, 63.24; H, 5.08; CH_3O, 34.31. UV λ_{max}^{EtOH} mμ (log ε) : 282 (4.62), 340 (4.87). (Luteolin 7,3,′4′-trimethyl ether : 277 (4.50), 340 (4.83)).

Acetylation of this substance afforded colorless needles, m.p. 179~180°, while ethylation with Et_2SO_4 afforded colorless needles, m.p. 151°, negative to $FeCl_3$ test.

ii) With $Me_2SO_4+K_2CO_3$: A mixture of 1 g. of pedalitin, 100 cc. of dehyd. Me_2CO, 4 g. of K_2CO_3, and 3 g. of Me_2SO_4 was boiled in an oil bath for 25 hr., cooled, and Me_2CO was distilled off. The residue was dissolved in a small quantity of MeOH and water was added in drops until a slight turbidity appeared. The mixture was warmed to dissolve the precipitate and allowed to stand, by which colorless prismatic crystals, m.p. 172~173°, were obtained, giving negative $FeCl_3$ test. Yield, 0.65 g. *Anal.* Calcd. for $C_{20}H_{20}O_7$: C, 64.51; H, 5.37; $5CH_3O$, 41.66. Found : C, 64.39; H, 5.57; CH_3O, 41.44.

Demethylation of Pedalitin—A mixture of pedalitin and HI (b.p. 125°) was boiled for 5 hr. in an oil bath at 160~165°, cooled, and poured into water. The yellow, gel-like precipitate was collected by filtration, washed with $NaHSO_3$ solution and water, and dried. Recrystallization from MeOH afforded yellow microneedles, m.p. over 325°, coloring dark brown to $FeCl_3$. *Anal.* Calcd. for $C_{15}H_{10}O_7 \cdot 2H_2O$: C, 53.25; H, 4.14; $2H_2O$, 10.65. Found : C, 53.22; H, 4.61; H_2O, 11.01.

Acetylation of this substance by the usual procedure gave colorless needles, m.p. 245~246°. *Anal.* Calcd. for $C_{25}H_{20}O_{12}$: C, 58.60; H, 3.93. Found : C, 58.54; H, 4.07.

Methylation of the demethylated product with Me_2SO_4 gave colorless prisms, m.p. 172~173°, undepressed on admixture with the product obtained by methylation of pedalitin with Me_2SO_4 and K_2CO_3 as described above.

Mixed fusion of the above acetate, m.p. 245~246°, with pedalitin acetate, m.p. 242~243°, showed m.p. 220°.

Alkali Fusion of Pedalitin—Two grams of pedalitin was added in small portions to a fused mixture (220~230°) of 20 g. of KOH and 2 cc. of H_2O, by which a vigorous effervescence occurred and the mixture turned brownish black. After cool, the mixture was dissolved in water, acidified with H_2SO_4, under cooling in ice, and extracted several times with Et_2O. The combined Et_2O extract was washed a few times with saturated $NaHCO_3$ solution. The alkali solution was acidified and shaken with Et_2O.

i) Phenolic portion : No residue on evaporation of Et_2O.

ii) Acid portion : Evaporation of Et_2O left brownish black solid which was recrystallized from hot water, forming colorless needles, m.p. 197°. Yield, 100 mg. The substance colored bright green to $FeCl_3$ and the color changed to rose red on addition of $NaHCO_3$. No depression of m.p. on admixture with protocatechuic acid, m.p. 197~198°.

Alkali Decomposition of Pedalitin—A solution of 1.2 g. of pedalitin in 5 g. of KOH and 12 cc. of H_2O was boiled for 100 min. in N_2 stream. After cool, the mixture was cooled in ice and acidified with 10 g. of 50% H_2SO_4, and K_2CO_3 thereby formed was removed by filtration. The filtrate was saturated with NaCl and extracted with Et_2O. The Et_2O extract was treated with $NaHCO_3$ solution as described in the preceding experiment.

i) Phenolic portion : The residue obtained on evaporation of Et_2O was negative to the pine-splinter reaction and colored bright green to $FeCl_3$. Recrystallization from benzene afforded colorless needles, m.p. 116~117°, undepressed on admixture with 3,4-dihydroxyacetophenone, m.p. 117°.

ii) Acid portion : Treated in the same manner as in the preceding experiment. Colorless needles, m.p. 196~197°, undepressed on admixture with protocatechuic acid, m.p. 197~198°.

Alkali Decomposition of Pedalitin Tetramethyl Ether—A mixture of 0.8 g. of pedalitin tetramethyl ether, m.p. 172~173°, and 45 cc. of 25% KOH was boiled for 6 hr. over a direct flame, by which pedalitin tetramethyl ether dissolved gradually. After cool, insoluble matter (0.3 g.) was filtered off, the filtrate was acidified with H_2SO_4 with cooling in ice, and extracted with Et_2O, and separated into acid and phenolic portions. Et_2O was evaporated from the phenolic portion, the residue was dissolved in a small quantity of benzene, and petr. ether (b.p. 40~50°) was added dropwise until appearance of slight turbidity. This was warmed to dissolve the precipitate and allowed to stand, from which colorless plates, m.p. 140~150°, separated. This colored dark brown to $FeCl_3$. Admixture with 3,4,5-trimethoxyphenol, m.p. 143°, showed no depression in m.p.

Position of the Methoxyl in Pedalitin—i) Alkaline decomposition of pedalitin trimethyl ether: A mixture of 0.5 g. of the trimethyl ether, m.p. 189~190°, and 20 cc. of 25% KOH was boiled for 2 hr. over a direct flame, cooled, and filtered. The filtrate was acidified with 20% H_2SO_4 and extracted with Et_2O. The phenolic portion obtained after evaporation of Et_2O was recrystallized from benzene, forming colorless needles, m.p. 76~77°, undepressed on admixture with 4,5-dimethoxyresorcinol, m.p. 76°. This substance colored reddish brown to $FeCl_3$ and violet to the pine-splinter test. *Anal.* Calcd. for $C_8H_{10}O_4 \cdot H_2O$: C, 51.06; H, 6.38; $2CH_3O$, 34.34; H_2O, 9.10. Found : C, 50.67; H, 6.412; CH_3O, 33.69; H_2O, 8.92.

The acid portion was recrystallized from hot water to form colorless needles, m.p. 176°, undepressed on admixture with veratric acid, m.p. 178~179°.

ii) Alkali decomposition of pedalitin trimethyl ether : A mixture of 0.5 g. of the trimethyl ether, m.p. 221~222°, and 20 cc. of 25% KOH was treated as above (i) but phenolic substance was not obtained. The acid portion afforded veratric acid as colorless needles, m.p. 176°.

iii) Ethylation and alkali decomposition of pedalitin trimethyl ethers (one, m.p. 189~190°, the other, m.p. 221~222°) : A mixture of 1.8 g. of the trimethyl ether, m.p. 189~190°, 6 g. of K_2CO_3, 3 g. of Et_2SO_4, and 130 cc. of dehyd. Me_2CO was refluxed for 28 hr. in an oil bath, cooled, and Me_2CO was distilled off. To this residue, 50 cc. of water was added, the mixture was extracted with Et_2O, and the syrupy residue was dissolved in 10 cc. of MeOH. On addition of 10 cc. of water to this solution and allowing it to stand, 0.8 g. of colorless needles, m.p. 150~151°, was obtained. This gave negative reaction to $FeCl_3$.

A mixture of 1.1 g. of the trimethyl ether of m.p. 221~222°, 4 g. of K_2CO_3, 3 g. of Et_2SO_4, and 100 cc. of Me_2CO was refluxed for 25 hr. and treated as above, affording 0.4 g. of colorless needles, m.p. 160°; negative to $FeCl_3$ test.

Alkali decomposition was carried out by heating each of the foregoing pedalitin trimethyl monoethyl ether (0.4 g.) and 45 cc. of 30% KOH for 6~8 hr. over a direct flame. Both were sparingly soluble. After cool, unreacted substance was filtered off, the filtrate was acidified with 20% H_2SO_4, and extracted with Et_2O. The Et_2O extract was washed with $NaHCO_3$ solution and evaporated, leaving a yellow, viscous substance. Attempted recrystallization from petr. ether was not successful. The substance colors pale purplish black to $FeCl_3$. Detail of its paper partition chromatography is given in the main text.

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Summary

A flavonoid, isolated from the leaves of sesame (*Sesamum indicum* L.), was determined as 5,3′,4′-trihydroxy-6-methoxyflavone 7-glucoside.

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