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Maackiain[1),*]

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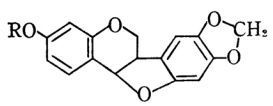
This isolation and determination of the structure of a new, naturally-occurring chromanocoumaran, maackiain (I) R=H, are described. On the basis of these results, the structure of pterocarpin is revised to (I) R=CH_3.



(I)

In preceding papers[2,3)] the isolation and the structure of a new isoflavanone, sophorol $C_{16}H_{12}O_6$, were discussed. Biogenetical considerations[2)] suggest the probable coexistence of pterocarpinoids

1) Oxygen Heterocycles. VII. Read before the 14th Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1961. A brief account of this work has been published. H. Suginome, *Experientia,* **18**, 161 (1962).

* It was suggested to call this class of naturally occurring substances pterocarpinoids.

2) H. Suginome, *Tetrahedron Letters,* **1960**, 16; and preceding paper.

3) H. Suginome, *J. Org. Chem.,* **24**, 1655 (1959).

or rotenoids in the same plant. Therefore, we decided to examine this problem. A previous paper[3] described the isolation of an unknown phenolic substance, which was accompanied by sophorol and which had the molecular formula $C_{16}H_{12}O_5$. In reference to the source of this new compound, the name "maackiain" is suggested. The following proof of the structure of the compound as a chromanocoumaran gives support to the earlier biogenetical suggestions.[2]

Maackiain formed leaflets containing a half-equivalent molecule of water from aqueous methanol. The analytical data of dried maackiain crystals (m. p. 180—181°C) are in excellent agreement with the molecular formula of $C_{16}H_{12}O_5$ as has already been reported.[3] The compound is optically active, giving a value of $[\alpha]_D^{20}$ 251.7° in a chloroform solution. The optical rotatory dispersion curve of maackiain exhibits a negative Cotton effect curve. This was phenolic. A Zeizel determination showed no methoxyl group to be present. The presence of a methylenedioxyl group in the maackiain molecule was, however, suggested by the positive color tests. Maackiain showed the following maxima in infrared absorption, confirming all of the chemical evidence for the functional groups in the maackiain molecule: (3472: phenolic hydroxyl) (1617, 1600, 1507: aromatic C=C) (1496, 1379, 1347, 1315, 1292, 1267, 1234, 1204, 1180) (1161: C-O in dihydropyran) (1150, 1125, 1112, 1078, 1064, 1016, 978). (1035, 929: $-C-O-CH_2-O-C-$) (888, 861, 844, 813, 781, 758, 740 cm^{-1}).

On the basis of the above evidence, the functional groups of maackiain may be summarized as follows:

$$C_{15}H_9 \begin{cases} -O- \\ -O- \\ -O-CH_2-O- \\ -OH \text{ (phenolic)} \end{cases}$$

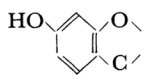
In accordance with the above results, maackiain readily formed mono-*O*-methyl ether, $C_{17}H_{14}O_5$, with ethereal diazomethane or with methyliodide and potassium carbonate in dry acetone. The *O*-methyl ether showed no band attributable to a hydroxyl stretching frequency in the infrared region.

In connection with a possible biogenetic linking of pterocarpin and homopterocarpin to naturally-occurring 2'-hydroxyisoflavonoids, the reduction of a carbonyl in the functional pattern (I) of sophorol, followed by dehydration to ether linkage, should give rise to a functional pattern (II) of maackiain.

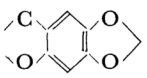
$$C_{14}H_8 \begin{cases} C=O \\ OH \text{ phenolic} \\ OH \text{ phenolic} \\ -O-CH_2-O- \\ -O- \end{cases} \xrightarrow[-H_2O]{+H_2} C_{15}H_9 \begin{cases} -O- \\ \text{——} \\ OH \text{ phenolic} \\ -O-CH_2-O- \\ -O- \end{cases}$$

(I) (II)

Furthermore, *O*-methylmaackiain has the same functional groups as pterocarpin. These considerations lead to the assumption that the two arylic systems in the maackiain molecule may be the A and B groupings, which are chromophores of sophorol:[2]



(A)



(B)

The ultraviolet spectra of maackiain and *O*-methylmaackiain in alcohol gave valuable information on the structure. Maackiain exhibits characteristic absorption peaks in two main regions, one of high intensity at 311 mμ (ε, 8510), and the other of a lower intensity at 286 mμ (ε, 5120). The medium intensities of these absorption maxima showed that the aromatic nucleus is in no conjugation with any other chromophore, e. g., ethylenic linkage.

Curves a, b, c, and d, in Fig. 1 show the ultraviolet absorption curves of 2:4-dimethoxyphenylacetonitrile, 2-methyl-4:5-methylenedioxyphenol, *O*-methylmaackiain and the summation of curves a and b respectively. As may be expected from the above discussion, curves c and d are very similar in shape.

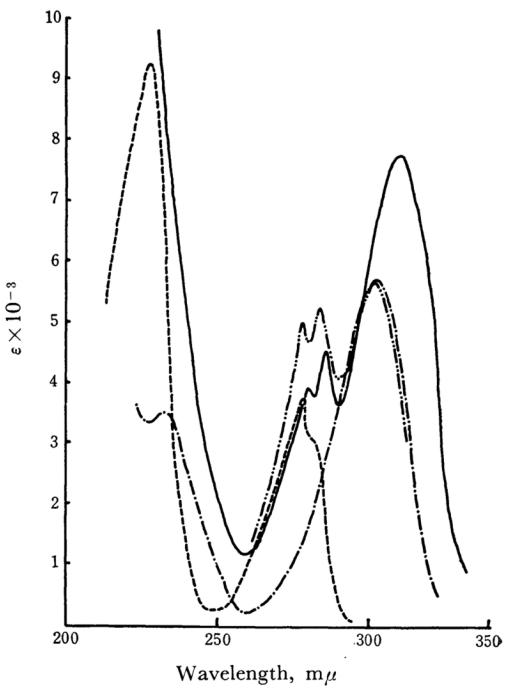
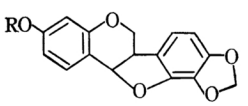


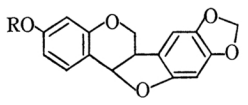
Fig. 1. UV spectra.
------ 2, 4-Dimethoxyphenylacetonitrile (curve a)
—·—· 2-Methyl-4: 5-methylenedioxyphenol (curve b)
——— *O*-Methylmaackiain (curve c)
—··— Curve a + b (curve d)

On the other hand, the ultraviolet absorption spectrum of *O*-methylmaackiain and that of pterocarpin** were also superimposable, and a mixed melting point determination of the two substances showed no depression.

Therefore, the structure of *O*-methylmaackiain was thought to be either III ($R=CH_3$) or IV ($R=CH_3$).



(III)

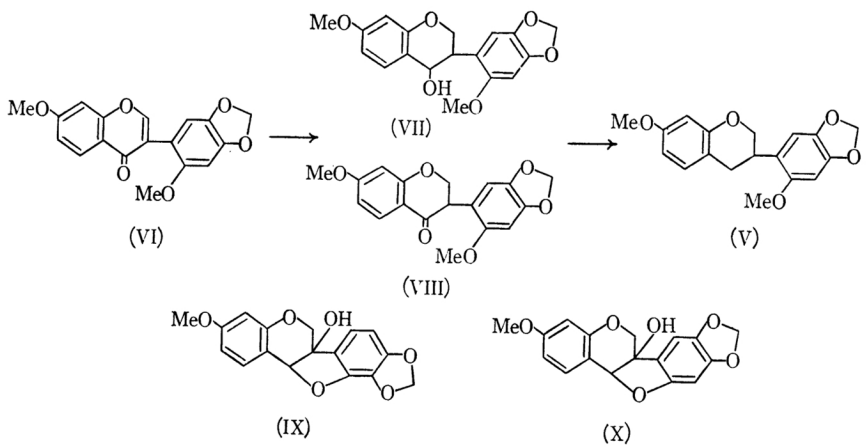


(IV)

Formula IV ($R=H$) for maackiain was proved as follows: the catalytic reduction of *O*-methylmaackiain gives rise to a new phenolic substance, $C_{17}H_{14}O_5$, melting at 146—148°C. The same substance was also produced in a good yield by the Birch reduction[4] of *O*-methylmaackiain. This phenol gave mono-*O*-methyl ether.

On the basis of formula IV, the structure of this phenolic compound should be explained by the isoflavan structure (V) formed by the hydrogenolysis of the benzilic-type ether linkage.

The structure of the *O*-methyl ether of dihydro-*O*-methylmaackiain was confirmed by the synthesis



(VI) (VII) (VIII) (V)

(IX)

(X)

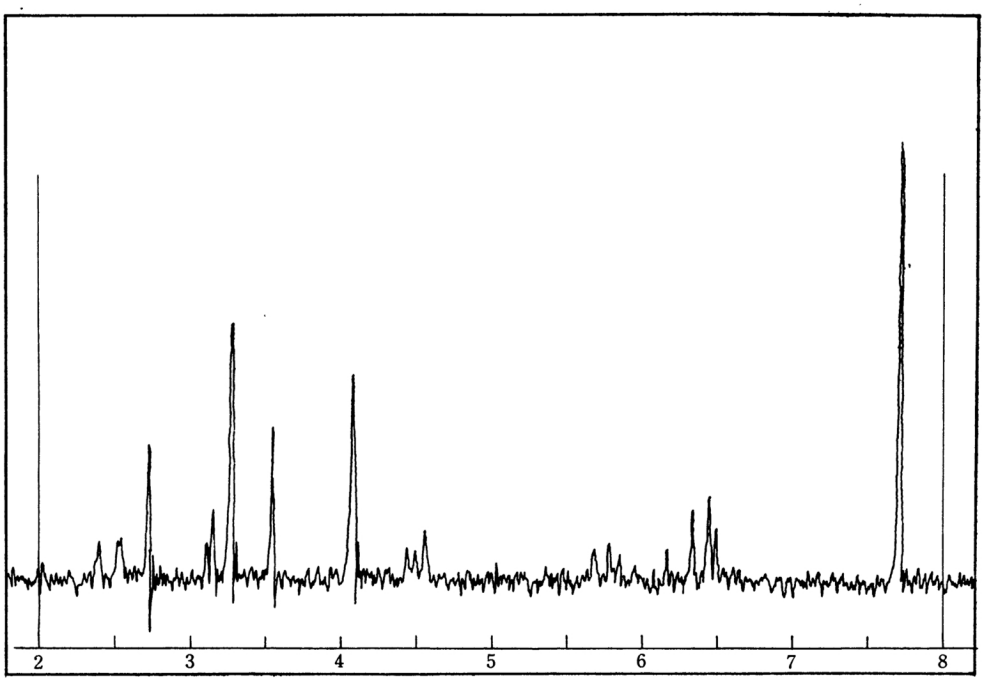


Fig. 2. NMR spectrum.

** Kindly provided by Dr. J. W. W. Morgan from the collection of Dr. F. E. King.

4) A. J. Birch, *Quart. Rev.*, **4**, 69 (1950).

of racemic 2': 7-dimethoxy-4': 5'-methylenedioxy-isoflavan (V, R=CH_3). The catalytic reduction of 2' :7-dimethoxy 4': 5'-methylene-dioxyisoflavone (VI)[3] gave an oily mixture of the corresponding isoflavan-4-ol (VII) and isoflavanone (VIII). The Clemmensen reduction of this gave racemic 2':7-dimethoxy - 4':5' - methylenedioxy - isoflavan (V), which had a m. p. of 111—113°C (V, R=CH_3).*** The latter and *O*-methyldihydro-*O*-methylmaackiain gave identical infrared-spectra in a chloroform solution, thus establishing the structure IV (R=H) for maackiain.[5] Since a comparison of the infrared spectra of *O*-methylmaackiain and pterocarpin in a chloroform solution disclosed that the two substances are identical, the structure of pterocarpin should be corrected to IV (R=CH_3). This finding is complementary to the conclusion, from the NMR spectrum, on the structure of pterocarpin.[6] Moreover, this conclusive evidence for the structure of pterocarpin and biogenetical considerations suggest that the structure of pisatin,[7] for which the structure IX has been given, should be X.

On the basis of a direct comparison with *O*-methylanhydrosophorol[3] and some spectral properties, the structure of pisatin was recently revised[8] to X. The NMR spectrum of *O*-acetylmaackiain (Fig. 2) further confirms the above conclusion.

The sharp single peak at $\tau=7.72$ which has the relative area of three hydrogens is due to the acetoxy group at the position 7. The value is in good agreement with the value of the acetoxy group attached to the aromatic ring. The peaks between $\tau=2.0$ and $\tau=3.6$ are attributed to aromatic protons. Ring A in *O*-acetylmaackiain has a pair of hydrogen atoms at the positions 5 and 6 and a hydrogen atom at the position 8. As expected, a hydrogen at the position 5 which is coupled with only one hydrogen on C6 appeared as a doublet centered at $\tau=2.44$. The coupling constant ($J=8.9$ c. p. s.) is an expected value for the ortho coupling of hydrogens on a benzene ring. Of the rest of the signals of this ABX system of the ring A, only one doublet, at $\tau=3.13$ and $\tau=3.16$, was observable. This doublet is considered to be a part of the quartet arising from a hydrogen on the position 6 which is coupled with a proton on the position 8 (meta coupling) and with a proton on the position 5 (ortho coupling). Other protons on the benzene ring B, will give two singlet peaks attributable to a proton on the position 3' and to a proton on the position 6' (para coupling). The lines at $\tau=3.56$ (1 proton) and $\tau=3.29$ (2.5 protons) are assigned to these protons. The observed broadening and the area of the former suggested that this had to include another proton. A proton on the position 8 will give rise to a doublet by meta coupling with a proton on the position 6. However, no isolated doublet was observed in the expected position. The peaks which are due to a hydrogen on the position 8 apparently formed a broad single peak at $\tau=3.29$, together with the signal of a hydrogen of the position 6'. It should be noted that, due to the *O*-acetyl substituent on the position 7, the position of the signal of a proton on the position 8 is shifted to a lower magnetic field than that to be expected for a proton adjacent to two oxygens. The peak at $\tau=3.29$ also includes the rest of the quartet of a proton on the position 6. The methylenedioxy peak appeared at 4.09 τ as a singlet. The triplets with center of 4.51 τ are assigned to a hydrogen on the position 4, which is coupled with only a proton of the position 3.

Since the interaction with a proton on the position 3 should give rise to doublets with the lines of a proton on the position 4, the following can explain the situation. The shape of the peaks indicates that they may be an unresolved quartet. *O*-Acetylmaackiain in deuteriochloroform could exist in an equilibrium of the two possible cis conformations, which will have somewhat different coupling constants for a proton on the position 4. The molecular model of the strainless configuration of *O*-acetylmaackiain, in which the two hydrogens on carbons 3 and 4 are cis, suggests that two conformations are possible. The conformational inversion between these two forms could be favorable in a chromanocoumaran system due to the lack of some 1, 3-hydrogen interaction. The two hydrogens on the position 2 which consititute the AB part of the ABX system and a hydrogen on the position 3 appeared in the range from $\tau=5.5$ to $\tau=6.6$. The two hydrogens on the position 2 should give rise to eight lines; these hydrogens appeared as a triplet centered at $\tau=6.42$. The rest of this ABX system, i. e., a hydrogen at the position 3, formed the multiplets $\tau=5.69$ to $\tau=6.17$.

*** A communication from the University of Manchester recorded m. p. 110—111°C for the same compound which was derived from pterocarpin.
C. W. L. Bevan, A. J. Birch, B. Moore and S. K. Mukerjee, *Tetrahedron Letters*, **1962**, 673.

5) After this work was completed, Professor Shibata of The University of Tokyo informed us that he and his colleague had isolated several chromanocoumarans from *Sophora subprostrata chun et T. Chen* and *Sophora japonica* L. By direct comparison, one of the chromanocoumarans was proved to be identical with maackiain. S. Shibata and Y. Nishikawa, *Chem. and Pharm. Bull.*, **11**, 167 (1963).
Furthermore, inermin isolated from *Andira inermis* H. B. K. by Cocker et al. is identical with maackiain. W. Cocker, T. Dahl, C. Dempsey and T. B. H. McMurry, *Chem. & Ind.*, **1962**, 216.

6) J. B. Bredenberg and J. N. Shoolery, *Tetrahedron Letters*, **1961**, 285.

7) D. R. Perrin and W. Bottomley, *Nature*, **191**, 76 (1961).

8) D. R. Perrin and W. Bottomley, *J. Am. Chem. Soc.*, **84**, 1919 (1962); D. D. Perrin and D. R. Perrin, ibid., **84**, 1922 (1962).

In view of the observed antifungicidal activity of this group of compounds, it is of interest to compare the variation in biological activity with the variation in structure. The results of tests on the antimicrobial activity[7] of maackiain and related compounds, kindly performed by Professor Yuji Sasaki and his colleagues, are recorded in the Experimental section.

Experimental[9]

The isolation and purification were described, with the isolation of sophorol, in the Experimental section of the previous paper.[3] Maackiain formed leaflets from aqueous methanol. After it had been dried under a vacuum, the melting point of this compound (178.5—179°C) rose to 180—181°C.

Found: C, 65.68; H. 4.69; 1/2H_2O, 3.7, 3.6. Calcd. for $C_{16}H_{12}O_5 \cdot 1/2H_2O$: C, 65.53; H, 4.46; 1/2$H_2O$; 3.6%. Found: (in a sample dried at 100—110°C in vacuo) C, 67.67; 67.33; H, 4.41; 4.58. Calcd. for $C_{16}H_{12}O_5$: C, 67.60; H, 4.26%.

$[\alpha]_D^{22}$ −251.7° (*c* 0.46, chloroform). RD[10] (*c* 0.074, methanol). 26°: [α]700 −175.8, [α]589 −227.0, [α]400 −559.0, [α]360 −684.0.

Maackiain was soluble in aqueous 2 N sodium hydroxide but insoluble in aqueous sodium hydrogen carbonate. Maackiain dissolved readily in acetone, ethylacetate, dioxane, chloroform and benzene. It gave no ferric reaction. The solution of maackiain in concentrated sulfuric acid became green when a drop of a 5% alcoholic gallic acid solution was added.

Maackiain-*O*-methyl Ether (Pterocarpin).—*a*) *With Diazomethane.*—Maackiain (150 mg.) in acetone (6 ml.) was treated with an ethereal solution of diazomethane (from nitrosomethylurea. 1.5 g.) and then set aside overnight. Evaporation under diminished pressure and the recrystallization of the crystalline residue from absolute ethanol yielded *O*-methylmaackiain, m. p. 168—169°C.

Found: C, 67.92; H. 4.74; OCH_3, 10.35. Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.37; OCH_3, 10.40%. RD[6] (*c* 0.077, chloroform), 16°:[α] 700 −119.4, [α]589 −223.5, [α]400 −530.1, [α]340 −481.0.

UV *max.*: 310 mμ (ε, 10156), 386 mμ (ε 5687), 280 mμ (ε, 4813).

This was insoluble in a 2 N sodium hydroxide solution. The solution of the compound in concentrated sulfuric acid became green when one drop of a 5% alcoholic gallic acid solution was added.

b) *With Methyl iodide.*—A mixture of maackiain (670-mg.), potassium carbonate (2 g.), methyl iodide (15-ml.), and acetone (14 ml.) was heated under reflux for 4 hr. The filtered solution was then evaporated, and the residue was washed with water and recrystallized from alcohol.

Colorless leaflets; m. p. 168—169°C (560 mg.).

Dihydro-*O*-methylmaackiain Obtained by the Hydrogenolysis of *O*-Methylmaackiain.—*a*) *By Catalytic Hydrogenation.*—*O*-Methylmaackiain (200 mg.), dissolved in glacial acetic acid (24 ml.) containing a pre-reduced platinum catalyst (platinum oxide, 40 mg.), was hydrogenated for 2 hr. at room temperature. After the catalyst had then been removed, the filtrate was evaporated to dryness. The residue was treated with aqueous 2 N sodium hydroxide. Upon the acidification of the sodium hydroxide solution, crude dihydro-*O*-methylmaackiain separated. This was recrystallized from aqueous ethanol, forming colorless leaflets (m. p. 146—148°C) (8 mg.). Crude *O*-methylmaackiain (158 mg.) was recovered unchanged.

Found: C, 68.10; H, 5.12. Calcd. for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37%.

IR spectrum: 3285 cm^{-1} (phenolic OH); 1618, 1591 and 1499 cm^{1-} (aromatic C=C).

UV max.: 290 and 302 mμ (ε, 6729 and 6467).

b) *With Sodium and Liquid Ammonia.*—Sodium (50 mg.) in small pieces was stirred into *O*-methylmaackiain (150 mg.) in toluene (8 ml.) and liquid ammonia (28 ml.) within a five-minute period. After the reaction mixture had been set aside overnight, 10 ml. of water was added. After the extraction of the mixture with ether (40 ml.), the ethereal solution was extracted with 4 N aqueous sodium hydroxide (20 ml.×2). The alkaline layer was acidified with 6 N hydrochloric acid and extracted with ether (40 ml.+20 ml.+20 ml.), washed with water, and dried. The solution was evaporated to dryness to yield an intractable residue (120 mg.). The neutral fraction, after it had been washed and dried, yielded a crystalline residue of 155 mg. This was recrystallized from dilute ethanol, yielding dihydro-*O*-methylmaackiain (m. p. 146—148°C, 67 mg.) which was identical with the specimen obtained from procedure a) above.

***O*-Methyl Ether of Dihydro-*O*-methylmaackiain.**—Dihydro-*O*-methylmaackiain (20 mg.), methyliodide (0.02 ml.), anhydrous potassium carbonate (60 mg.), and dry acetone (1 ml.) were refluxed for 4 hr. After the potassium carbonate had been removed, the filtrate was evaporated to dryness and the residue was washed with a small volume of water and then recrystallized from aqueous ethanol, yielding *O*-methyl ether as short needles, m. p. 109—110°C.

Found: C, 68.66; H, 5.70. Calcd. for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77%.

***O*-Acetylmaackiain.**—Maackiain (85 mg.) was dissolved in 0.5 ml. of pyridine and 0.5 ml. of acetic anhydride and then set aside. A work-up by the standard procedure gave crude *O*-acetylmaackiain (93 mg.). This was recrystallized from aqueous ethanol. M. p. 177—178°C; leaflets; 60 mg.

Found: C, 66.37; H, 4.29. Calcd. for $C_{18}H_{14}O_6$: C, 66.25; H, 4.32%.

The Hydrogenation of 2′ : 7-Dimethoxy-4′ : 5′-methylenedioxyisoflavone.—When isoflavone (84 mg.) in acetic acid (20 ml.) was hydrogenated in the presence of a pre-reduced platinum catalyst (PtO_2, 20 mg.) for 26 min., it absorbed 1.1 mol. of hydrogen. The solvent was then removed under reduced pressure to yield 77 mg. of a residue, which showed hydroxyl and γ-pyranone carbonyl in its infrared spectrum and

9) Melting points are uncorrected. Ultraviolet spectra were taken in ethanol unless otherwise stated. Analyses were made by Mrs. Noriko Etoh in this laboratory and Mr. Kusuo Narita, Department of Pharmacy, Faculty of Medicine, Hokkaido University. The NMR spectrum was taken by Japan electron optics 3H 60 high resolution NMR spectrometer (60 Mc.). Tetramethylsilane was used as internal standard.

10) Measurements by Dr. Masao Maruyama of Tohoku University.

which was immediately submitted to the next reduction.

The Clemmensen Reduction of the Mixture of 7 : 2′-Dimethoxy 4′ : 5′-methylenedioxyisoflavan-4-ol and 7 : 2′-Dimethoxy-4′ : 5′-methylenedioxyisoflavanone.—The mixture (77 mg.) of isoflavan-4-ol and isoflavanone obtained above was dissolved in 2 ml. of toluene. To this solution was then added mercury amalgam, which had been prepared from zinc (0.79 g.), mercuric chloride (0.087 g.), concentrated hydrochloric acid (3 ml.), and water (1.5 ml.). The solution was refluxed for 14 hr. and 20 min. To the solution further amounts of hydrochloric acid (0.12 ml. × 3) were then added after 2 hr. 40 min., 6 hr. 5 min., and 9 hr. 15 min. The zinc amalgam was filtered and washed with toluene-ether. To the combined filtrate and washings, there was added a further volume of ether. The ethereal solution was washed with water, a 2 N sodium hydroxide solution (5 ml.) and twice again with water (5 ml. × 2) successively. The solution was then dried over sodium sulfate. On evaporation, the residual yellow oil crystallized. M. p. 96—107°C; 50 mg. This was recrystallized from ethanol to yield d, *l*-7: 2′-dimethoxy-4′: 5′-methylenedioxyisoflavan, m. p. 111—113°C.

Found: C, 68.66; H, 5.86. Calcd. for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77%.

2-Methyl-4 : 5-methylenedioxyphenol.—A mixture of zinc dust (5 g.), 0.5 g. of mercuric chloride, 0.25 ml. of concentrated hydrochloric acid, and 7.5 ml. of water was stirred for 15 min. The aqueous solution was decanted, and the zinc amalgam was covered with 5 ml. of water and 5 ml. of concentrated hydrochloric acid. To this refluxed solution, there was then added 0.5 g. of sesamol in 2.5 ml. of concentrated hydrochloric acid and 2.5 ml. of water over the course of 1 hr. and twenty minutes. After a further 2 ml. of concentrated hydrochloric acid had been added, the solution was refluxed for 2 hr. The solution was then cooled to room temperature. The aqueous layer was separated from the zinc amalgam, and sodium chloride was added to the solution. This was extracted with ether. The ethereal solution was combined with the ethereal washings of the zinc amalgam and washed with an aqueous sodium chloride solution, decolorized with activated charcoal, and dried. After the ether had been removed, the residue (0.36 g.) was distilled under reduced pressure (2.5 mmHg) (bath temp. 132—142°C) (0.172 g.). This distillate was recrystallized from petroleum benzine. 96 mg. M. p. 58—59°C. IR: 3573 cm^{-1} (OH) (chloroform) UV λ_{max} (ε_{max}): 232 (3525) 302 (5720).

Antimicrobial Tests (Performed by Professor Yuji Sasaki and his colleagues, Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Faculty of Agriculture, by courtesy of Professor Tōshi Irie).—The inhibition of the growth of bacteria, fungi or yeast was assayed according to the agar-plate method. The compounds were introduced in the form of acetone dilutions or in the form of crystals on agar. The strains of bacteria, fungi or yeasts used are described in Table I. These were incubated on the agar medium at the optimum temperature.

TABLE I.

		Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	*Saccharomyces cerevisiae*	*Candida albicans*	*Aspergillus niger*	*Aspergillus fumigatus*	*Penicillium roqueforti*
a)	2′-Hydroxyisoflavanone	−	−	−	−	−	−	−	−
b)		−	−	−	−	−	−	−	−
a)	Sophorol	−	−	−	−	−	−	−	−
b)		−	−	−	−	±	−	−	−
a)	Maackiain	−	−	−	++	++	++	++	++
b)		−	−	−	−	−	−	−	−
a)	Pseudobaptigenin	−	−	−	−	−	−	−	−
b)		−	−	−	−	−	−	−	−

a) acetone solution b) crystals

When the compounds were introduced in the form of crystals, no antimicrobial activity was observed in any of the specimens. However, in an acetone solution, maackiain exhibited antimicrobial action in reaction to yeast and fungi.

The author is grateful to Professors Tōshi Irie, Takeshi Matsumoto and Tadashi Masamune for their kind interest in this work, and Drs. F. E. King and J. W. W. Morgan, for providing the sample of pterocarpin. The author wishes to thank Mr. Toshiro Kio for help in experiment and Mr. Hanzo Shimokawa for the measurements of NMR spectra.