stirring at room temperature for five hours. The ether was evaporated and the azo compound was crystallized from ethanol, 5.8 g., 70% yield, m.p. 110–112° dec. (reported m.p. 110–112°).

*p*-**Nitrophenyl-azo-triphenylmethane.**[5]—*p*-Nitrophenylhydrazine, 15.3 g. (0.1 mole) was treated with 14 g. (0.05 mole) of trityl chloride as described above. The mixture was filtered and concentrated, diluted with chloroform and treated directly with saturated bicarbonate solution and excess 30% hydrogen peroxide as described above. The azo compound was crystallized from methanol and obtained in over-all yield of 47%, 9.2 g., m.p. 118–119° dec. (reported m.p. 118.5°).

*p*-**Tolyl-azo-triphenylmethane.**[5]—*p*-Toluidine (18 g.) was diazotized by treatment with hydrochloric acid and sodium nitrite, and the diazonium compound was reduced with stannous chloride, leading to *p*-tolylhydrazine hydrochloride. This was isolated and converted to *p*-tolylhydrazine, 7.5 g., 37% yield, m.p. 61–65°. Treatment with 8.6 g. of trityl chloride and oxidation with hydrogen peroxide as described for the unsubstituted compound led to N-*p*-tolyl-N′-tritylhydrazine, 7.5 g., 66% yield, m.p. 155° (from ethanol), and then to the azo compound, 62% yield, m.p. 103° dec. (reported m.p. 103.5°).

*p*-Bromo**phenyl-azo-triphenylmethane.**—*p*-Bromophenylhydrazine hydrochloride, 22.3 g. (0.1 mole) was treated with sodium hydroxide and extracted with ether. The ether solution was dried over potassium hydroxide, treated with 0.05 mole of trityl chloride in the usual way, filtered and oxidized directly with hydrogen peroxide, leading to the azo compound in over-all yield of 30%, m.p. 114–115° dec.

*Anal.* Calcd. for $C_{25}H_{16}N_2Br$: C, 70.3; H, 4.48. Found: C, 70.3; H, 4.55.

*p*-**Acetaminophenyl-azo-triphenylmethane.**—*p*-Aminoacetanilide, 25 g. (Matheson Co.) was converted to *p*-acetaminophenylhydrazine hydrochloride, 18.5 g. Attempts to use the free base, or the hydrochloride, neutralized by alkali, in ethanol in the next stage were unsuccessful. The hydrazine hydrochloride, 5 g., and 7 g. of trityl chloride were dissolved in 80 ml. of pyridine, which had been dried over potassium hydroxide, and allowed to react at room temperature for two hours. The mixture was filtered and the filtrate was diluted with water until it became turbid. A product slowly solidified; it was crystallized from ethanol, 8 g., 80% yield, m.p. 122 dec., presumably the N-*p*-acetaminophenyl-N′-tritylhydrazine. The hydrazine, 4 g., was dissolved in 1:1 acetone–alcohol and oxidized in the usual way. The azo compound was crystallized from ethanol, 3 g., 73% yield, m.p. 112–113° dec.

*Anal.* Calcd. for $C_{27}H_{23}N_3O$: N, 10.36. Found: N, 10.35.

*p*-**Methoxyphenyl-azo-triphenylmethane.**[6]—*p*-Anisidine (25 g.) was diazotized and reduced to *p*-methoxyphenylhydrazine hydrochloride; this was isolated and converted to the free base, m.p. 65°. The hydrazine, 5 g., was dissolved in 20 ml. of dried pyridine and treated with a solution of 10 g. of trityl chloride in 40 ml. of dried pyridine under nitrogen, first at 0°, then at 25° for one hour. The mixture was diluted with ether, washed with water, hydrochloric acid and water, dried, concentrated, triturated with 95% ethanol and cooled. A yellow solid was isolated, m.p. 80–90° dec., which was dissolved in chloroform and oxidized with hydrogen peroxide at 0° for one hour. The chloroform was evaporated and the residue, 9 g., was crystallized rapidly from ethanol, leading to the azo compound, 5.3 g., 41% yield, m.p. 113–114° dec. (reported m.p. 114°).

**Triphenylmethylhydrazine.**[22]—Trityl chloride, 0.075 mole, was treated with 68% hydrazine hydrate, 0.16 mole, in ether. The mixture was warmed gently and filtered, and the filtrate treated with hydrogen chloride gas, leading to crude tritylhydrazine hydrochloride, 87% yield, melting variously in different runs from 105–115° to 130–140° (reported m.p. 108–113°).

*p*-**Hydroxyphenyl-azo-triphenylmethane.**—Triphenylmethylhydrazine hydrochloride, 12 g., m.p. 130–140°, was dissolved in 50 cc. of 95% ethanol and added at 0° to a solution of quinone (freshly crystallized from benzene, m.p. 113–115°) in 100 cc. of 95% ethanol. The solution was diluted with 10 volume per cent. of water and kept at 4° for 24 hours. The mixture was filtered, the filtrate concentrated in vacuum, the residue taken up in ether, washed repeatedly with water, dried over magnesium sulfate, concentrated in vacuum, dissolved in 95% ethanol, treated with a little water and cooled. A yellow solid, 4.5 g., m.p. 70–80°, was collected and crystallized several times from 3:1 alcohol–water, leading to the azo compound, m.p. 115–116° dec., 3.2 g., 23% yield.

*Anal.* Calcd. for $C_{25}H_{20}N_2O$: C, 82.4; H, 5.53. Found: C, 82.5; H, 5.45.

**Acknowledgment.**—We are pleased to acknowledge generous support of this work by Frederick Gardner Cottrell Grants of Research Corporation and by an allocation from the Committee on Research Grants of Brandeis University.

(22) J. Stieglitz, THIS JOURNAL, **38**, 2720 (1916).

WALTHAM, MASSACHUSETTS

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[CONTRIBUTION FROM THE GOVERNMENT FOREST EXPERIMENT STATION OF JAPAN]

# Two New Flavonoid Glycosides from the Leaves of *Phellodendron amurense* Ruprecht

BY MASAO HASEGAWA AND TERUO SHIRATO

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From the fresh leaves of *Phellodendron amurense*, a tree of *Rutaceae*, two new flavonoid glycosides have been isolated. One of them, phellamurin, is shown to be 5,7,4′-tetrahydroxy-8-(γ-hydroxyisovaleryl)-flavanonol-7-glucoside, and the other, amurensin, to be the corresponding flavonol glucoside. The conversion of phellamurin into amurensin has been successfully achieved.

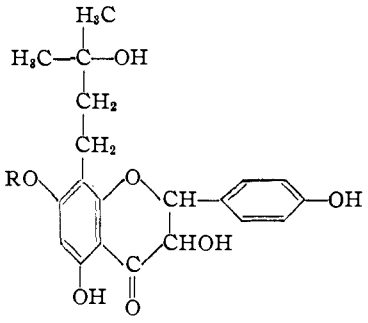
## Introduction

*Phellodendron amurense* Ruprecht is a tree widely distributed in mountainous regions of the central and northern part of Japan, and when adult usually attains a height of 20 m. From the water soluble portion of the methanolic extract of the leaves of this tree, a colorless flavanonol glycoside has been obtained, and from the water-insoluble portion, a yellow flavonol glycoside. These two substances are new to the literature, and the name phellamurin for the former glycoside and the name amurensin for the latter have been proposed.

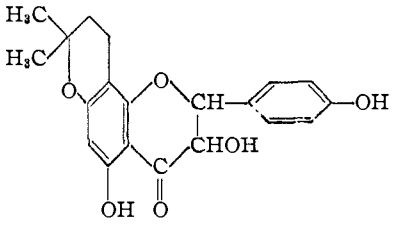
The structures of phellamurin and amurensin (Fig. 1) have been established, utilizing in part the study of the relationship of these new compounds with the compounds icariin, nor-icariin and β-anhydroicaritin (Fig. 2), whose structures had previously been established by Akai and coworkers.[1] These established interrelationships are shown in Fig. 3.

Details of the isolation, properties and proof of structure of the compounds phellamurin and amur-

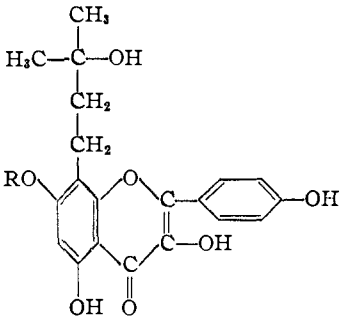
(1) S. Akai and K. Nakagawa, *J. Pharm. Soc. Japan*, **55**, 155 (1935); S. Akai, M. Imaida and T. Matsukawa, *ibid.*, **55**, 214 (1935).



Phellamurin (R = glucose)

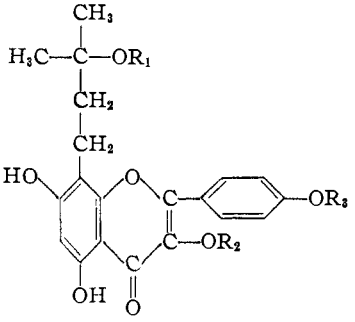


Phellamuretin

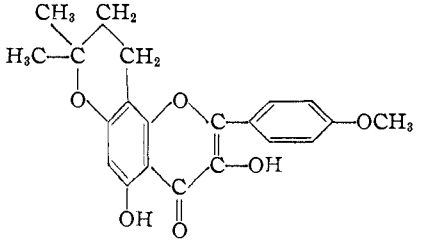


Amurensin (R = glucose)

Fig. 1.



Icariin ($R_1$ = glucose, $R_2$ = rhamnose, $R_3$ = $CH_3$)
Nor-icariin ($R_1$ = glucose, $R_2$ = rhamnose, $R_3$ = H)



β-Anhydroicaritin

Fig. 2.

ensin are reported in the experimental portions below.

## Experimental

All melting points are not corrected.

**Isolation and Purification of Phellamurin and Amurensin.**—One charge of fresh leaves of *Phellodendron amurense* was extracted with 3 l. of boiling methanol for 3 hr., and the extraction was repeated again with new methanol. From the combined filtered extract, methanol was distilled off *in vacuo* on a water-bath, and the residue mixed with 2 l. of water. After heating for a time, the whole was filtered, and the residue was once more extracted with 1 l. of hot water. The combined filtrate was decolorized while hot with a small amount of charcoal, and the filtered solution was let stand overnight. A gelatinous mass which separated in the solution was filtered and treated with 1 l. of hot water. The portion insoluble in hot water was filtered off, and the filtrate was mixed with an equal volume of ethyl acetate. Crystals of phellamurin, which had deposited after standing overnight, were recrystallized repeatedly from ethyl acetate containing a small volume of water. The phellamurin separated in long colorless needles of m.p. 205°. The yield of phellamurin from 5 kg. of fresh leaves was about 50 g.

The portion insoluble in hot water was dried and washed with ether, and was recrystallized from a large volume of methanol. Amurensin was thus obtained as minute yellow needles of m.p. 290°. The yield was 2.2 g.

**Phellamurin.**—A methanolic solution of phellamurin gives a violet coloration when reduced with zinc powder and concentrated hydrochloric acid, and a reddish violet coloration with magnesium powder and concentrated hydrochloric acid. It also produces a green ferric chloride color.

The latter coloration is just the same as that given by the crude extract of the leaves. This coloration has been considered as characteristic of the flavanonols.[2] The characteristic feature of the absorption spectra also coincides with that of flavanonols such as katuranin, taxifolin (distylin) and ampeloptin[3] ($\lambda_{max}$ 290 mμ). The absorption spectra of flavanones will be dealt with later elsewhere by the authors. Katuranin has the constitution 5,7,4′-trihydroxyflavanonol. (Recently, by private communication to the authors, Dr. J. Gripenberg of the Finland Institute of Technology wrote that Dr. Cohen at Sydney, Australia, has confirmed the structure of aromadendrin, which had been isolated from *Eucalyptus* species many years ago, to be the same as katuranin. In view of this fact, the name katuranin might be redundant.)

Phellamurin is insoluble in benzene, ether, petroleum ether, ligroin, cold water and cold ethyl acetate. It is readily soluble in methanol, ethanol and acetone.

Absorption: $\lambda_{max}$ 290 mμ, log ε 4.24; $\lambda_{max}$ 345 mμ, log ε 3.60; $\lambda_{min}$ 322 mμ, log ε 3.30. *Anal.* Calcd. for $C_{28}H_{32}O_{12}$: C, 58.20; H, 5.98. Found: C, 58.05; H, 5.95.

**Phellamurin Dimethyl Ether.**—Four grams of phellamurin, dissolved in 100 ml. of acetone, was heated 1 hr. with 10 g. of potassium carbonate and 2 ml. of dimethyl sulfate. A small portion of it then gave no color reaction with ferric chloride. After filtering, acetone was removed by distillation and the residue washed with ether. The dimethyl ether (3 g.) crystallized from methanol in colorless needles of m.p. 200°.

*Anal.* Calcd. for $C_{28}H_{30}O_{10}(OCH_3)_2$: C, 59.57; H, 6.38; $OCH_3$, 10.99. Found: C, 59.99; H, 6.35; $OCH_3$, 11.33.

**Phellamurin Acetate.**—Two-tenths g. of phellamurin was treated with 1 ml. each of pyridine and of acetic anhydride for 24 hr. in the cold. The mixture was poured into cold water, and the precipitate was recrystallized from methanol. The acetate consists of colorless slender prisms of m.p. 202°; yield 0.2 g.

*Anal.* Calcd. for $C_{42}H_{48}O_{20}$: C, 57.79; H, 5.50. Found: C, 58.87; H, 5.16.

**Hydrolysis of Phellamurin.**—1.11 g. of phellamurin was heated in 40 ml. of 5% sulfuric acid for 3 hr. on a water-bath. The deposited white mass of the aglycone phellamuretin was separated by filtration and then recrystallized from methanol.

The yield of crude phellamuretin was 0.7 g. This crude mass was repeatedly recrystallized from methanol, and colorless needles of m.p. 220° were obtained.

After extracting with ether, the sugar remaining in the mother liquor was estimated by means of Bertrand's method (404 mg., 408 mg. as glucose). The residual solution was carefully neutralized with barium carbonate, filtered and evaporated on a boiling water-bath to a small quantity, and then filtered. From this, phenylglucosazone (m.p. 208°) was prepared by the usual method. By paper chromatography, no other sugar besides glucose could be detected.

(2) J. C. Pew, THIS JOURNAL, **70**, 3031 (1948).

(3) T. Kubota, *Ann.*, **544** (1940), 253 mμ. In this reference, the name "ampelopsin" was used throughout, but the name ampeloptin is the correct one according to the private communication of Dr. Kubota.

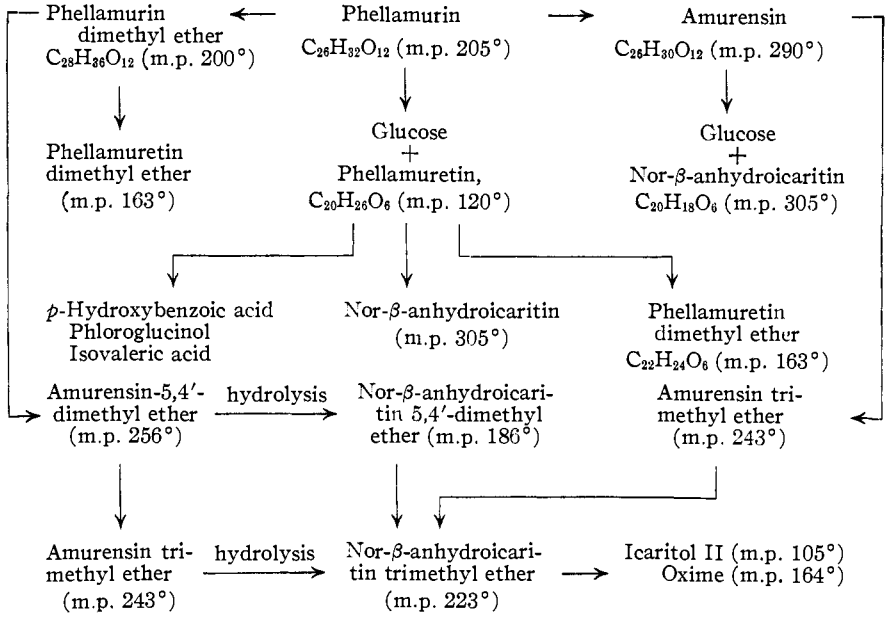



Fig. 3.

**Phellamuretin.**—A methanolic solution gives a purplish brown coloration with ferric chloride. When reduced with magnesium or zinc powder and concentrated hydrochloric acid, a reddish purple coloration was developed.

It is insoluble in benzene, petroleum ether, chloroform and cold ethyl acetate, sparingly soluble in ether, and readily soluble in acetone, methanol and ethanol.

Absorption: $\lambda_{max}$ 300 mμ, log ε 4.28; $\lambda_{min}$ 255 mμ, log ε 3.17. *Anal.* Calcd. for $C_{20}H_{20}O_6$: C, 67.41; H, 5.61. Found: C, 67.12; H, 5.56.

The analysis gave a somewhat different value than the expected one ($C_{20}H_{22}O_7$), but rather it coincided with $C_{20}H_{20}O_6$. This indicated the probable loss of 1 mol of water on heating in an acidic medium.

In 1935, Akai, *et al.*,[1] isolated from the leaves and roots of a small herb, *Epimedium micranthum* (Berberidaceae) two flavonol glycosides, icariin ($C_{33}H_{42}O_{16}\cdot 1\frac{1}{2}H_2O$) and nor-icariin ($C_{32}H_{40}O_{16}\cdot 3H_2O$) (see Fig. 2). He found that the aglycone icaritin ($C_{20}H_{22}O_7$) gave rise to β-anhydroicaritin (see Fig. 2), with loss of 1 mol of water, when it was heated with 20% hydrochloric acid or with 5% ethanolic sulfuric acid (80% ethanol) or heated above its melting point (about 250–260°). He also described that on hydrolysis with 3% sulfuric acid, nor-icariin gave nor-icariside (I) and rhamnose, and that the former of these products, on hydrolysis with 50% sulfuric acid, gave nor-β-anhydroicaritin and glucose.

**Phellamuretin Dimethyl Ether.**—A mixture of 0.5 g. of phellamuretin, 100 ml. of acetone, dimethyl sulfate and 6 g. of potassium carbonate was heated on a water-bath. After one hour's heating, no coloration with ferric chloride was observed. The solvent was then removed by distillation and the residue was mixed with a small amount of petroleum ether with stirring. The resulting solid was filtered; yield 0.3 g. This was recrystallized from methanol. These crystals consisted of prisms with m.p. 163°.

*Anal.* Calcd. for $C_{20}H_{18}O_4(OCH_3)_2$: $OCH_3$, 16.14. Found: $OCH_3$, 16.25.

**Phellamuretin Monomethyl Ether.**—Into a suspension of 0.1 g. of phellamuretin in 50 ml. of ether, 100 ml. of an ethereal diazomethane solution was added and the solution was allowed to stand overnight. The ether was evaporated and the residue crystallized from methanol to give needles of m.p. 187°. This product gives a purplish brown coloration with ferric chloride and an orange coloration with magnesium powder and concentrated hydrochloric acid.

*Anal.* Calcd. for $C_{20}H_{19}O_5(OCH_3)$: $OCH_3$, 8.32. Found: $OCH_3$, 7.85.

**Fusion of Phellamuretin with Potassium Hydroxide.**—One and one-half grams of phellamuretin, 30 g. of potassium hydroxide and 1 ml. of water were heated in a nickel crucible over a direct flame at 200° for 10 minutes, at 205° for 8 minutes, and then at 250–270° for 10 minutes. After cooling, the resulting solid was dissolved in 200 ml. of water. Under cooling, 10% sulfuric acid was added until the solution reacted acidic against litmus paper. Then the solution was distilled with steam. The distillate, saturated with sodium chloride, was obtained. A half ml. of this oily substance and 8 ml. of aniline were made to react in a sealed tube at 200° for 3 hr. on an oil-bath. The reaction mixture was poured into 200 ml. of 5% hydrochloric acid solution and after standing overnight the precipitate was recrystallized from diluted methanol. Prisms of m.p. 114° were obtained. The yield was 0.15 g. The melting point on admixture with authentic isovaleric anilide did not show any depression, but on admixture with acetanilide, the melting point fell to 96°.

*Anal.* Calcd. for $C_{11}H_{15}ON$: N, 7.90. Found: N, 8.18.

The mother liquor was extracted several times with ether, and then the ethereal layer was extracted with 1% sodium bicarbonate solution and washed with water. After acidification with sulfuric acid, the bicarbonate solution was then extracted with ether. Then, after evaporation of the ether, the residue was recrystallized from water. Colorless needles of m.p. 210° were obtained. The melting point showed no depression, when the crystals were mixed with authentic *p*-hydroxybenzoic acid. The ethereal layer was then extracted with 1% potassium hydroxide solution and the ether gave a residue, which was recrystallized from a small amount of water. These crystals consisted of prisms of m.p. 212°. The substance proved to be identical with phloroglucinol as found by the mixed melting test, as well as through paper chromatography.

**Phellamuretin Acetate.**—Two-tenths g. of phellamuretin, 2 ml. of acetic anhydride and one drop of concentrated sulfuric acid was made to react at room temperature, and the resulting transparent solution was poured into cold water. By recrystallization of the resulting solid from methanol, the acetate of phellamuretin was obtained in colorless needles of m.p. 199°.

**Dimethyl Phellamuretin Acetate.**—Two-tenths g. of phellamuretin dimethyl ether was acetylated by the ordinary method; yield 0.2 g. Recrystallized from methanol, it was obtained in colorless prisms of m.p. 177°.

*Anal.* Calcd. for $C_{20}H_{17}O_4(OCH_3)_2(CH_3CO)$: $OCH_3$, 14.55. Found: $OCH_3$, 14.64.

**Oxidation of Phellamuretin.** (1) By **Oyamada's[4] Method.**—1.8 g. of phellamuretin was dissolved in 40 ml. of methanol with 5 ml. of 10% potassium hydroxide and 1 ml. of perhydrol and kept in an ice-box for 24 hr.; 80 ml. of water was then added. The precipitate (1.5 g.) was recrystallized from methanol. Minute yellow needles of m.p. 305° were obtained. These were found to be identical with nor-β-anhydroicaritin (m.p. 305°) by mixed melting point analysis.

(2) By **Another Method.**—Five g. of phellamuretin was dissolved in 50 ml. of 10% potassium hydroxide solution and boiled about 4 minutes. After cooling, the black precipitate was separated by filtration and recrystallized from methanol; minute yellow needles of m.p. 305° with a yield of 0.3 g. were obtained. This substance is identical with nor-β-anhydroicaritin. The melting points of its acetate and that of its methyl ether were 212 and 223°, respectively.

*Anal.* (of the methyl ether). Calcd. for $C_{20}H_{15}O_6(OCH_3)_3$: $OCH_3$, 23.43. Found: $OCH_3$, 23.29. *Anal.* (of the acetate). Calcd. for $C_{20}H_{15}O_6(CH_3CO)_3$: C, 65.00; H, 5.00. Found: C, —; H, 4.94.

(4) T. Oyamada, *J. Chem. Soc. Japan*, **55**, 755 (1934).

**Oxidation of Phellamuretin Dimethyl Ether.**—One-half g. of phellamuretin dimethyl ether was treated by Oyamada's method to give nor-β-anhydro-icaritin dimethyl ether. The yield was 0.3 g. The product was recrystallized from methanol and obtained in yellow needles of m.p. 186°.

*Anal.* Calcd. for $C_{20}H_{16}O_4(OCH_3)_2$: $OCH_3$, 16.23. Found: $OCH_3$, 15.36.

Its methyl ether, m.p. 223°, on mixed melting point analysis, proved to be identical with Akai's β-anhydroicaritin dimethyl ether. This latter sample was sent to us through the courtesy of Dr. K. Maeda of the Hospital Dispensary of the University of Okayama from the original preparations of Dr. Akai.

**Alkali Decomposition of β-Anhydroicaritin Dimethyl Ether.**—One and one-half g. of this substance was decomposed by Akai's method,[5] and from the reaction products, *p*-methoxybenzoic acid and icaritol (II) (m.p. 105°) were obtained with yields of 0.3 and 0.5 g., respectively.

The melting point of the icaritol (II) oxime was 164°.

*Anal.* (of the icaritol II oxime). Calcd. for $C_{15}H_{21}O_5N$: N, 4.75. Found: N, 4.88.

According to Akai, icaritol (II) has the structure of 2-dimethyl-5-hydroxy-6-(ω-methoxyacetyl)-7-methoxychromane. The melting point of the icaritol (II) obtained here and its oxime coincided with those given by Akai for these compounds, respectively.

**Oxidation of Phellamurin Dimethyl Ether.**—Seven-tenths g. of phellamurin dimethyl ether was satisfactorily converted into amurensin 5,4'-dimethyl ether with a yield of 0.5 g. It crystallized out from methanol in needles of m.p. 256°. A methanolic solution gave a brown color with ferric chloride.

*Anal.* Calcd. for $C_{26}H_{30}O_{10}(OCH_3)_2$: $OCH_3$, 10.68. Found: $OCH_3$, 11.03. Absorption: $\lambda_{max}$ 365 mμ, log ε 4.42; $\lambda_{max}$ 265 mμ, log ε 4.42; $\lambda_{min}$ 290 mμ, log ε 4.08.

Its absorption spectrum indicates the probability of its flavonol nature.[6] The methyl ether of amurensin trimethyl ether has a melting point of 243°, which was identical with amurensin trimethyl ether (m.p. 243°). The identity was confirmed by mixed melting point test.

**Oxidation of Phellamurin.**—Five g. of phellamurin was treated by Oyamada's method. The yield was 3.0 g., of a yellow flavonol glycoside of m.p. 290°, which is amurensin itself. This was confirmed by mixed melting point determination. Amurensin ($C_{26}H_{30}O_{12}$) is thus a dehydrated product of phellamurin. Since the number of carbon atoms was unchanged, it might be concluded that the dehydration undoubtedly took place at the 2-3 position, as was ascertained in several other cases.[7]

From these results, one might consider that a free hydroxyl group is left at the 3-position in phellamurin. Accordingly, the glucose residue in phellamurin is regarded as located either at the 7-position or on the isovaleryl group. If the glucose residue lies on the isovaleryl group, the completely methylated phellamurin should possess three methoxyl groups, but such was not the case. So the position of the glucose residue must be 7.

Of the twelve oxygen atoms of phellamurin, five belong to the glucose residue, and six to the flavanonol. The twelfth one perhaps lies on the 5-C side chain, as a tertiary alcoholic group. If such an alcoholic group is assumed to be located at the third carbon atom, water would be easily lost between it and the hydroxyl group ortho to the detached 5-C chain, to give a chromane structure.

For this consideration, an effective support might be that the absorption band of phellamuretin in methanol ($\lambda_{max}$ 300 mμ) is shifted to longer waves about 10 mμ more than ordinary flavanonol and resembles that of a chromane[8] derivative. The results of the analysis of phellamuretin also agree with this fact and with these considerations.

**Hydrolysis of Amurensin 5,4'-Dimethyl Ether.**—Two-tenths g. of amurensin 5,4'-dimethyl ether was heated with 70 ml. of acetone and 70 ml. of 3% hydrochloric acid solution on a water-bath. Acetone was evaporated gradually. After 1.5 hr., yellow crystals were filtered and extracted with ether. The ether soluble portion, recrystallized from methanol, was obtained in yellow needles of m.p. 186°.

*Anal.* Calcd. for $C_{20}H_{16}O_4(OCH_3)_2$: $OCH_3$, 16.06. Found: $OCH_3$, 15.22.

The melting point of its monomethyl ether was 223°, and it is identical with β-anhydroicaritin dimethyl ether (m.p. 223°), as proved by mixed melting point determination.

**Amurensin.**—It gives a green coloration in methanolic solution with ferric chloride, and an orange coloration with magnesium powder and concentrated hydrochloric acid. It is sparingly soluble in the usual organic solvents except acetone, in which it is moderately soluble. The melting point of amurensin, recrystallized from a large volume of methanol, is 290°.

*Anal.* Calcd. for $C_{26}H_{30}O_{12}$: C, 58.42; H, 5.61. Found: C, 58.40; H, 5.52. Absorption: $\lambda_{max}$ 377 mμ, log ε 4.23; $\lambda_{max}$ 270 mμ, log ε 4.28; $\lambda_{min}$ 306 mμ, log ε 3.94.

**Hydrolysis of Amurensin.**—A suspension was made from 1 g. of amurensin and 20 ml. of water, and then 20 ml. of concentrated sulfuric acid was added drop by drop. It was neutralized under cooling with 10% potassium hydroxide solution, and the precipitate was filtered and recrystallized from methanol. The nor-β-anhydroicaritin was obtained in yellow needles of m.p. 305°; yield 0.32 g. 0.237 g. of amurensin gave 0.1602 g. of nor-β-anhydroicaritin. $C_{26}H_{30}O_{12} \rightarrow C_{20}H_{18}O_6 + C_6H_{12}O_6$. Calcd.: 66.4. Found: 67.5.

The mother liquor from the hydrolysis was neutralized with barium carbonate and the filtrate was evaporated to a sirup. The phenylosazone was prepared by the ordinary method. The melting point of the phenylosazone was 208° and no depression was observed when mixed with authentic glucosazone.

**Nor-β-anhydroicaritin.**—A methanolic solution gives a greenish brown coloration with ferric chloride. This compound is insoluble in the usual organic solvents, except acetone. The melting point is 305°.

*Anal.* Calcd. for $C_{20}H_{18}O_6$: C, 67.79; H, 5.08. Found: C, 67.72; H, 4.94. Absorption: $\lambda_{max}$ 365 mμ, log ε 4.32; $\lambda_{max}$ 271 mμ, log ε 4.38; $\lambda_{min}$ 296 mμ, log ε 3.90.

**β-Anhydroicaritin Dimethyl Ether.**—Two-tenths g. of nor-β-anhydroicaritin, 2 ml. of dimethyl sulfate, 10 g. of potassium carbonate and 100 ml. of acetone were heated on a water-bath for 6 hr. After filtering, the acetone was evaporated and the residue was recrystallized from methanol. The dimethyl ether was obtained in needles of m.p. 223°; yield 0.1 g.

*Anal.* Calcd. for $C_{20}H_{15}O_3(OCH_3)_3$: $OCH_3$, 23.43. Found: $OCH_3$, 24.17.

**Nor-β-anhydroicaritin Acetate.**—The acetate was prepared from nor-β-anhydroicaritin by the ordinary method; m.p. 212°.

**Amurensin Trimethyl Ether.**—Three-tenths g. of amurensin, 3 ml. of dimethyl sulfate, 12 g. of potassium carbonate and 50 ml. of acetone were heated on a water-bath for 1 hr. After evaporation of the methanol, the solution was mixed with 50 ml. of water and extracted several times with ether. After evaporation of the ether, the residue was recrystallized from methanol and the methyl ether was obtained in long needles of m.p. 223°; yield 0.1 g. The substance is identical with β-anhydroicaritin dimethyl ether as shown by mixed melting point determination.

**Amurensin Acetate.**—One-tenth g. of amurensin was treated with 1 ml. of pyridine and 2 ml. of acetic anhydride in the cold. The reaction mixture was poured into water after standing for a night. The resulting solid was recrystallized from methanol, and the acetate was obtained in long colorless prisms of m.p. 199°; yield 0.1 g.

*Anal.* Calcd. for $C_{42}H_{44}O_{19}$: C, 57.97; H, 5.31. Found: C, 58.67; H, 5.76.

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# 5-Ethyl-5-(1-methyl-3-carboxypropyl)-2-barbituric Acid and its Thio Analog. Metabolites from Pentobarbital and Thiopental

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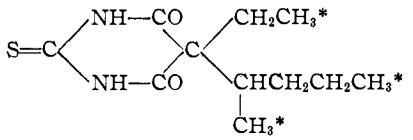
5-Ethyl-5-(1-methyl-3-carboxypropyl)-2-thiobarbituric acid has been synthesized and has been found identical with a metabolic product isolated from human urine after the administration of thiopental. The preparation of 5-ethyl-5-(1-methylpentenyl-4)-2-thiobarbituric acid and 5-ethyl-5-(1-methylbutenyl-3)-2-thiobarbituric acid is also described; these thiobarbiturates were prepared for metabolism studies. 5-Ethyl-5-(1-methyl-3-carboxypropyl)-barbituric acid has been synthesized and shown to be identical with a metabolite isolated from the urine of dogs receiving pentobarbital.

5-Ethyl-5-(1-methylbutyl)-2-thiobarbituric acid[1] is widely used in intravenous anesthesia. It is usually classified as an ultra-short-acting barbiturate; the studies of Brodie[2] on the physiological disposition and chemical transformation of thiopental in the body show that this thiobarbiturate is almost completely metabolized in man at a relatively slow rate following extensive localization in fat. From the urine of humans after intravenous administration of thiopental it was possible to isolate a metabolic product of the drug. This compound was evidently a thiobarbiturate, since its ultraviolet absorption spectrum was qualitatively identical with that of thiopental. The analysis and infrared spectrum indicated that a terminal methyl group had been converted to a carboxylic acid group; this was supported by the electrometric titration data ($pK_a$ 5.2 and 8.2). There are three possible positions for the carboxylic acid group, corresponding to the three terminal methyl groups of thiopental, as indicated by asterisks in the structure. Of these three possibilities, it was considered




most likely that oxidation occurred at the terminal position most distant from the quaternary carbon atom. This view[2] was dictated in part by the fact that 5,5-diethylbarbituric acid is largely excreted in unchanged form, and by the observations of Maynert[3] on the metabolic transformation of 5-ethyl-5-(1-methylbutyl)-barbituric acid (pentobarbital or Nembutal) into an oxidized product containing an hydroxyl group in the 3-position of the 1-methylbutyl chain.

5-Ethyl-5-(1-methyl-3-carboxypropyl)-2-thiobarbituric acid was synthesized by the method shown in Chart I. Diethyl (1-methyl-3-carboxypropyl)-ethylmalonate (VII) was obtained by ozonolysis of diethyl (1-methylpentenyl-4)-ethylmalonate (III). The initial product of the ozonolysis was the corresponding aldehyde, and this was oxidized by permanganate to the acid. The condensation of the acid ester VII with thiourea occurred under normal conditions although in low yield. The desired product VIII was isolated by countercurrent distribution. It was a colorless crystalline solid whose ultraviolet and infrared spectra were identical with those of the thiopental metabolite previously isolated from human urine.[2] A mixed melting point was not depressed. Paper chromatography experiments indicated that both substances (isolated and synthetic) were homogeneous and had the same $R_f$ value.[4]

This synthesis therefore establishes the structure of a metabolic product of thiopental.

The oxidation of a terminal methyl group of a hydrocarbon chain, which occurs in the body in this instance, may be related to the analogous reaction of $\omega$-oxidation of fatty acids.[5] The intermediate steps in a transformation of this kind are not known, but one possible route from a methyl group to a carboxylic acid group involves dehydrogenation to a terminal methylene group, followed by hydration to a primary alcohol and subsequent conversion of the alcohol to an acid.[6] Other possibilities include the direct introduction of an hydroxyl group, followed by further oxidation. To test the dehydrogenation hypothesis, and to determine the metabolic fate of the compounds, two thiobarbiturates containing a chain with a terminal methylene group were prepared. These were 5-ethyl-5-(1-methylpentenyl-4)-2-thiobarbituric acid and 5-ethyl-5-(1-methylbutenyl-3)-2-thiobarbituric acid. The latter compound corresponds to a hypothetical intermediate in the oxidation of thiopental.

In order to study the generality of $\omega$-oxidation in barbiturate metabolism in related compounds, Titus and co-workers[7] have studied the fate of pentobarbital in the dog. A barbituric acid with a terminal methyl group oxidized to a carboxyl group was iso-

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