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# 69. Shoji Shibata,*1 Tadakazu Murata,*2 and Mitiiti Fujita*1 : Studies on the Constituents of Japanese and Chinese Crude Drugs. X.*3 Wistin, A New Isoflavone Glucoside of *Wistaria* Spp.

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Otto[1] isolated a poisonous glycoside, named wistarin, m.p. 204°, from the bark of *Wistaria sinensis*, and described that it gave yellow coloration with alkali, violet to bluish green color with ferric chloride and white precipitates with lead sub-acetate. On hydrolysis with sulfuric acid wistarin yielded glucose and aglycone, whose structure, however, was not further elucidated.

Carelli *et al.*[2] obtained *n*-heptacosane, two optically inactive and low melting substances, and 22,23-dihydrostigmasterol from the flowers of *W. sinensis*.

The present authors have studied the principles of the roots, bark and heartwood of *W. floribunda* DC. (violet flowering) which is dextrorse growing wildly in the mountain district of Kanto.

A glucoside was obtained from the methanolic extract of the fresh bark in the yield of 0.1%, and the aglycone of the glucoside was isolated from the ether-soluble fraction in the yield of 0.005%.

The glucoside content in the roots and heartwood was low.

The glucoside, m.p. 209~210°, $[\alpha]_D^{12}$ −67.15° (c=1.43, acetic acid), has now been named wistin, and showed that it is represented by the formula, $C_{23}H_{24}O_{10}\cdot H_2O$. On acetylation, it yielded tetraacetate $C_{31}H_{32}O_{14}$, m.p. 165~166°. Hydrolysis of wistin with dil. sulfuric acid or emulsin afforded aglycone, $C_{17}H_{14}O_5$, m.p. 228~229°, and D-glucose. The ultraviolet spectra of wistin and its aglycone suggested that these compounds belong to isoflavone group.

The methyl ether of aglycone of wistin was boiled with 5% alcoholic potash to form $C_{17}H_{18}O_5$, m.p. 99~100°, which was proved to be identical with synthetic 2′-hydroxy-2-(*p*-methoxyphenyl)-4′,5′-dimethoxyacetophenone (III).

Oxidation of the aglycone with alkaline hydrogen-peroxide afforded anisic acid (IV) in a good yield, which would be derived from the side phenyl grouping of isoflavone.

Consequently, the aglycone of wistin must be represented as 6,4′-dimethoxy-7-hydroxy- or 7,4′-dimethoxy-6-hydroxyisoflavone.

According to McMurry and Theng[3] an isoflavone, named afromosin, isolated from a West African leguminoceous plant, *Afromosia elata* HARMS, was formulated as 7-hydroxy-6,4′-dimethoxyisoflavone (II). The identity of afromosin and the aglycone of wistin has been established by the mixed fusion of their acetates, m.p. 165~166° and 168~169° (mixed m.p. 167~169°) and by the comparison of infrared spectra. Thus, it has been established that wistin is afromosin-D-glucoside (I).

The paper chromatographical investigation showed that the bark of sinistrous *W. brachybotrys* SIEB. et ZUCC. f. *alba* OHWI (Japanese name : Shirafuji) and the horticultural white and violet flowering variations of *Wistaria* also contain wistin.

From the methanolic extract of the heartwood of *W. floribunda* DC. afromosin, wistin and an unknown substance were proved by the paper chromatography.

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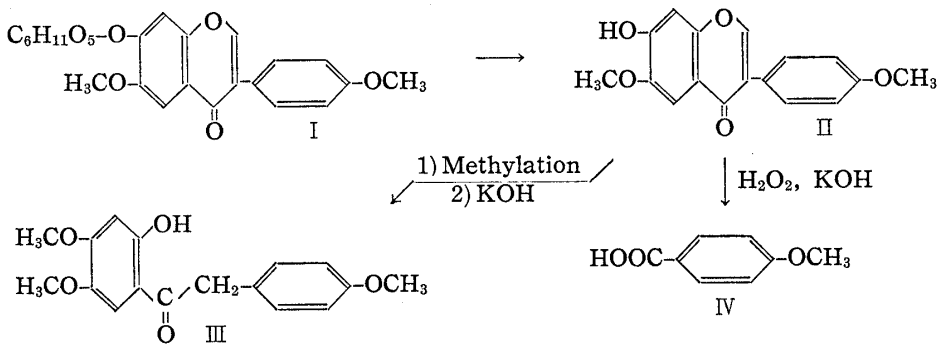
2) V. Carelli, P. Marchini, M. Ziffers, A. Breccia : Ann. Chim. (Rome), **46**, 1016 (1956) (C. A. **51**, 6664$^f$).
3) T. B. H. McMurry, C. Y. Theng : J. Chem. Soc., **1960**, 1491.

Contrary to the description of wistarin by Otto, wistin showed no remarkable poisonous effect in animals. The colorations of wistarin with ferric chloride and lead acetate recorded by the earlier worker were different from those of wistin.




## Experimental

**Extraction of the Bark of *Wistaria floribunda* DC.**——The fresh barks (9.5 kg.) of *W. floribunda* DC. were extracted three times with boiling MeOH.

The precipitates separated out on cooling were removed by filtration and washed with MeOH. The filtrate and washing were combined together and concentrated *in vacuo* to 5 L.

Treating the extract with $(AcO)_2Pb$, and the precipitates formed were removed by filtration, and the filtrate was concentrated to obtain a yellowish suspensions, which were extracted with $Et_2O$ and then with AcOEt, successively.

**Wistin (I)**——On concentration of the above AcOEt-extract, crude crystalline wistin separated out, which was recrystallized from MeOH and then from aq. MeOH or aq. $Me_2CO$ to form colorless needles m.p. 209~210°, yield, 10 g. (0.1%).

The paper chromatogram was developed by a mixture of BuOH-AcOH-$H_2O$ (4:1:5, upper layer) as the solvent and sprayed with $HIO_4$ solution and then with $KMnO_4$ solution. The spot was also detectable by its blue fluorescence under UV-illumination. Rf : 0.79. Wistin is insoluble in $Et_2O$, benzene, and $CHCl_3$, while it is soluble in $H_2O$, EtOH, MeOH, and $Me_2CO$, on heating. It does not give any coloration with $FeCl_3$, dil. NaOH, or Mg-HCl. It exhibits yellow color with Zn-HCl, Na-Hg, HCl and diazonium reagent. UV $\lambda_{max}^{95\%\,EtOH}$ mμ (log ε) : 261 (4.38), 320 (3.84); IR $\nu_{max}^{Nujol}$ $cm^{-1}$ : 3400, 1636, 1612, 1585. *Anal.* Calcd. for $C_{23}H_{24}O_{10}\cdot H_2O$ : C, 57.74; H, 5.48. Found : C, 57.73; H, 5.24.

**Afromosin (II)**——The above ethereal extract was concentrated and then shaken with 2*N* $Na_2CO_3$. The acidified aqueous layer was shaken again with $Et_2O$. Crude crystals were obtained from the ethereal extract on evaporation, which were recrystallized from EtOH to give colorless needles, m.p. 228~229°. Yield, 0.5 g. (0.005%, calculated from the weight of fresh bark used).

This is identical with the hydrolyzed product of wistin.

**Wistin Acetate**——Acetylation of wistin (50 mg.) with $Ac_2O$ (1 cc.) and pyridine (1.5 cc.) at room temperature afforded needles, m.p. 165~166° on recrystallization from EtOH. UV $\lambda_{max}^{95\%\,EtOH}$ mμ (log ε) : 261 (4.48), 321 (3.93). *Anal.* Calcd. for $C_{31}H_{32}O_{14}$ : C, 59.23; H, 5.13. Found : C, 59.53; H, 5.20.

**Hydrolysis of Wistin (Formation of Afromosin and D-Glucose)**——a) Wistin (2 g.) was dissolved in 2.5% $H_2SO_4$ (200 cc.) by warming on a boiling water bath, and after 2 hr. faintly yellowish precipitates separated out which were removed by filtration and dried (yield, 1.2 g.). Colorless needles, m.p. 228~229°, were obtained by recrystallization from EtOH. It dissolved not readily in EtOH, MeOH, and $Me_2CO$, but was sparingly soluble in $Et_2O$, benzene, $CHCl_3$ and $H_2O$. It gives no coloration with $FeCl_3$, yellow color with dil. NaOH, Zn-HCl, Na-Hg-HCl. The yellow color exhibited with Mg-HCl changes into pink on standing. With diazonium reagent it shows red color and the colorless solution in conc. $H_2SO_4$ turns into pink. UV $\lambda_{max}^{95\%\,EtOH}$ mμ (log ε) : 259 (4.42), 322 (4.02).

A bathochromic shift of the lower wave-length band which is generally observed in 7-hydroxyisoflavones when measured in 95% EtOH saturated with AcONa was not shown by this compound, while it gave 26 mμ bathochromic shift at the higher wave-length band ($\lambda_{max}$ 348 mμ). IR $\nu_{max}^{Nujol}$ $cm^{-1}$ : 3200, 1633, 1610, 1584. Rf : (BuOH-benzene-$(NH_4)_2CO_3$ buffer) 0.50, (BuOH-AcOH-$H_2O$ (4:1:5) 0.95. *Anal.* Calcd. for $C_{17}H_{14}O_5$ : C, 68.45; H, 4.73; 2 $CH_3O$, 20.8. Found : C, 68.17; H, 4.76; $CH_3O$, 20.2.

The acidic filtrate separated from the aglycone was neutralized with $BaCO_3$ and concentrated. The residual solution was paper chromatographically tested to prove the presence of D-glucose. Rf : (BuOH-AcOH-$H_2O$ (4:1:5)) 0.26; (Phenol-$H_2O$ (3:1)) 0.42. The authentic sample of D-glucose showed the same Rf-value.

The phenylosazone was prepared by the usual method to prove the identity with glucophenylosazone, m.p. 207~208° (decomp.) by a mixed fusion. Glucophenyltriazol, m.p. 194~195°, was derived from the phenylosazone by heating with $CuSO_4$.

b) Wistin (10 mg.) was dissolved in $H_2O$ (10 cc.) by heating and then the solution of emulsin (8 mg. in 3 cc.) was added. The mixture was allowed to stand for 24 hr. at 37° when white precipitates formed, in which afromosin was proved paper chromatographically. The supernatant of the centrifuged reaction mixture was concentrated and added with MeOH to precipitate enzyme. The presence of D-glucose was proved in the concentrated filtrate.

**Afromosin Acetate**——On acetylation with $Ac_2O$ and pyridine the aglycone of wistin afforded needles, m.p. 168~169°, which were proved to be identical with afromosin acetate by a mixed fusion with the authentic sample kindly supplied by Dr. McMurry. *Anal.* Calcd. for $C_{19}H_{16}O_6$: C, 67.05; H, 4.74. Found: C, 67.30; H, 4.78. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1766, 1648, 1610.

**Afromosin Methyl Ether**——The aglycone of wistin (950 mg.) was methylated boiling for 8 hr. with MeI (2.5 cc.), and anhyd. $K_2CO_3$ (1.2 g.) in $Me_2CO$ (100 cc.). The product was recrystallized from EtOH to form colorless needles, m.p. 178°. Yield, 600 mg. UV $\lambda_{max}^{95\%\ EtOH}$ mμ (log ε): 260 (4.45), 318 (4.02). *Anal.* Calcd. for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16; $3CH_3O$, 30.0. Found: C, 69.39; H, 5.16; $CH_3O$, 30.2.

**Oxidative Degradation of Afromosin (Formation of *p*-Anisic Acid)**——To an aglycone of wistin (= Afromosin) (300 mg.) dissolved in 10% KOH (15 cc.) was added 30% $H_2O_2$ (15 cc.) under ice cooling, and mixture was allowed to stand for 24 hr.

The excess of $H_2O_2$ was decomposed by the addition of $MnO_2$, and the filtrate was neutralized with dil. $H_2SO_4$. The ethereal extract of the reaction mixture was shaken with 5% $NaHCO_3$ to separate an acidic portion, from which colorless needles, m.p. 181~180° (yield, 90 mg.) was isolated. It was identified with *p*-anisic acid by a mixed fusion and comparison of the IR-spectra.

**Alkaline Degradation of Afromosin (Formation of 2′-Hydroxy-2-(*p*-methoxyphenyl)-4′,5′-dimethoxyacetophenone and *p*-Methoxyphenylacetic Acid)**——The aglycone of wistin (=afromosin) (150 mg.) was mixed with 10% KOH (5 cc.) and $H_2O$ (10 cc.), and the mixture was refluxed for 3 hr. under $N_2$ stream.

The cooled reaction mixture was saturated with $CO_2$ gas, and extracted with $Et_2O$ to isolate an oily substance (100 mg·), which was recrystallized from benzene to form faintly yellow needles, m.p. 127°. It gave a green color with $FeCl_3$. UV $\lambda_{max}^{95\%\ EtOH}$ mμ (log ε): 239 (4.05), 282 (4.03), 347 (3.96). *Anal.* Calcd. for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59. Found: C, 66.79; H, 5.53.

On methylation with MeI and $K_2CO_3$ in $Me_2CO$, the alkaline degradation product described above afforded 2′-hydroxy-2-(*p*-methoxyphenyl)-4′,5′-dimethoxyacetophenone (III) m.p. 99~100°.

Thus the above product, m.p. 127° was proved to be 2′,4′-dihydroxy-2-(*p*-methoxyphenyl)-5′-methoxyacetophenone.

The remainning $CO_2$ saturated aqueous layer of the $Et_2O$ extraction mentioned above was acidified with dil. HCl and shaken again with $Et_2O$.

Leaflets, m.p. 85~86° (from petr. ether) were obtained from the $Et_2O$ extracts, which were proved as being *p*-methoxyphenylacetic acid by a mixed fusion with the synthetic sample.

**Alkaline Degradation of Afromosin Methyl Ether (Formation of 2′-Hydroxy-2-(*p*-methoxyphenyl)-4′,5′-dimethoxyacetophenone (III))**——A mixture of methyl ether of aglycone of wistin (600 mg.) and 5% ethanolic KOH (40 cc.) was refluxed. After cooling the reaction mixture was diluted with $H_2O$ and then neutralized with dil. HCl, when yellowish needles separated out, which were recrystallized from EtOH to give colorless needles or leaflets, m.p. 99~100°. It shows a green coloration with $FeCl_3$. On admixture with synthetic 2′-hydroxy-2-(*p*-methoxyphenyl)-4′,5′-dimethoxyacetophenone, it showed no depression of melting point. UV $\lambda_{max}^{95\%\ EtOH}$ mμ (log ε): 277 (4.10), 344 (3.94). IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 1622, 1602. *Anal.* Calcd. for $C_{19}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.75; H, 6.06.

**2′,4′,5′-Trihydroxy-2-(*p*-methoxyphenyl)acetophenone**——Hydroxyhydroquinone (1.8 g.), *p*-methoxybenzyl cyanide (2.2 g.) and anhyd. $ZnCl_2$ (1.5 g.) were mixed in anhyd. $Et_2O$ (40 cc.), and dried HCl gas was introduced for 2 hr. under cooling at 0°. The original green color of solution changed into dark violet.

The reaction mixture was set aside in a refrigerator for 40 hr. The solvent was removed and the remaining syrupy ketimine hydrochloride was washed with $Et_2O$ and then added with $H_2O$ (90 cc.) and a few drops of conc. $H_2SO_4$. The solution was boiled for 1 hr., and the crystals separated out after cooling were recrystallized from aq. EtOH to form yellow needles, m.p. 180~181°.

It gives green color with $FeCl_3$ and yellow with aq. NaOH. UV $\lambda_{max}^{95\%\ EtOH}$ mμ (log ε): 242 (4.04), 283 (4.03), 352 (3.93). *Anal.* Calcd. for $C_{15}H_{14}O_5$: C, 65.68; H, 5.14. Found: C, 65.56; H, 5.13.

**2′-Hydroxy-2-(*p*-methoxyphenyl)-4′,5′-dimethoxyacetophenone**——A mixture of the above synthesized ketone (120 mg.), MeI (0.4 cc.), anhyd. $K_2CO_3$ (0.9 g.) in $Me_2CO$ (5 cc.) was refluxed for 1.5 hr. The product was treated in the usual manner to recrystallize from EtOH. 2′-Hydroxy-2-(*p*-methoxyphenyl)-4′,5′-dimethoxyacetophenone shows m.p. 99~100°, and a green coloration with $FeCl_3$. *Anal.* Calcd· for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.65; H, 6.02.

**Extraction of the Wood Part of *Wistaria floribunda* DC.**——The dried wooden part of *W. floribunda* (3.7 kg.) was extracted 3 times with MeOH. The methanolic extract was concentrated to 5 L. and treated with $(AcO)_2Pb$ and lead subacetate, successively. The filtrate was treated with $H_2S$ to remove PbS, and extracted with $Et_2O$ and AcOEt as described previously for the bark extract. Thus wistin was obtained in the yield of 0.003%.

From the precipitates formed by the addition of lead subacetate, a brownish syrup (19.5 g.) was isolated, which gave 3 fluorescent spots (Rf 0.95, 0.80, 0.45) under UV-illumination, on the paper chromatogram developed with BuOH-AcOH-$H_2O$ (4:1:5). The upper two spots represented afromosin and wistin, respectively.

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## Summary

A new glucoside, $C_{23}H_{24}O_{10}\cdot H_2O$, m.p. 209~210°, $[\alpha]_D^{12}$ −67.15° (c=1.43, acetic acid) was isolated from *Wistaria floribunda* DC. and some allied plants, and named wistin. The aglycone of wistin was proved to be identical with afromosin (=7-hydroxy-6,4′-dimethoxyisoflavone (II)), which had been isolated by McMurry *et al.* from *Afromosia elata* HARMS. Wistin was formulated as in I.

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## *70.* Sadao Iguchi, Atsuko Inoue, and Chieko Kurahashi: Studies on Pyrone Derivatives. (IX).*1 On the Reaction of Dehydroacetic Acid to the Primary Amines and Ammonia. (1).

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Dehydroacetic acid (DHA) is one of the officially recognized food-preservatives in Japan and also used frequently as an antiseptic in medicinal preparations. Therefore, we have been investigating its chemical behaviors with special interest. In previous papers,[1~4] it has been reported that DHA was reactive with ammonia, primary amines and some of the compounds possessing amino radical, such as amino acids and sulfanilamides, and Schiff's base type compounds were readily produced at first by the reaction between them in the solution. Such reaction of DHA was not always stopped at the step of the formation of Schiff's base, but it tended to proceed secondly to the transformation to pyridone derivatives even under mild conditions in some cases. The latter fact was

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*1 Part VIII, This Bulletin: **10**, 1070 (1962).
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1) S. Iguchi, *et al.*: Yakugaku Zasshi, **77**, 1258 (1957).
2) *Idem*: This Bulletin., **7**, 323 (1959).
3) *Idem*: *Ibid.*, **8**, 1 (1960).
4) *Idem*: *Ibid.*, **9**, 1016 (1961).