

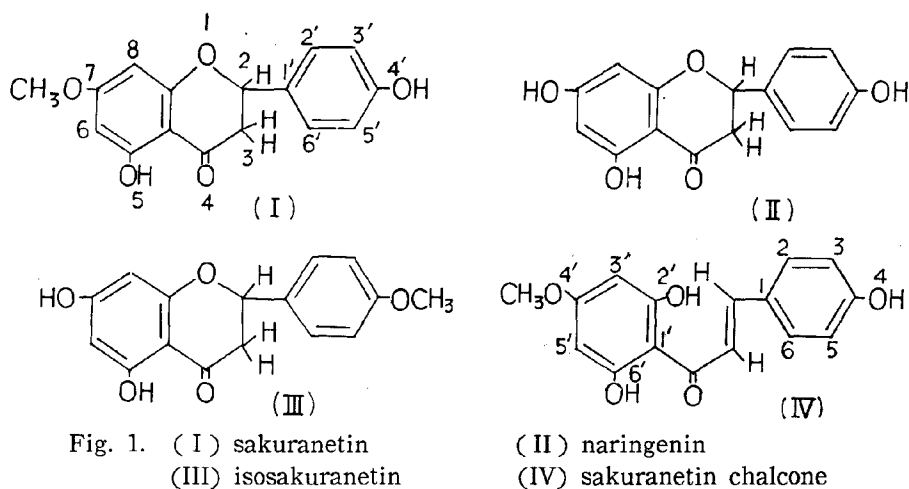
Studies on Wood Phenolics (III)*, **

Identification of Sakuranetin from Wood of *Haplormosia monophylla* HARMS (*Leguminosae*)

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佐藤 惺***・林 良興***・北尾弘一郎***：木材フェノール性化合物に
関する研究（第3報）*Haplormosia* 材からサクラネチンの同定

An African wood, IDEWA¹⁾ (*Haplormosia monophylla* HARMS) grown in Gabon is imported in our country, and used for furniture and veneer industries. It resembles *Afrormosia* spp. or teak in appearance. This report is concerned with the finding of sakuranetin and (I) sakuranetin chalcone (IV) in this wood. Sakuranetin (4', 5-dihydroxy-7-methoxyflavanone) has been found from a few kinds of trees which belong to *Prunus* spp. (bark and wood)^{2,3)}, *Eucalyptus* sp. (kino)⁴⁾ and *Juglans* sp. (bark)⁵⁾.



Major parts of ether soluble material was predicted to belong to so-called "phenolic compounds," because eighty percents of the ether extracts was soluble in (A) 5 % sodium carbonate and (B) 5 % sodium hydroxide solution.

From fractions (A) and (B), four components (Rf value—0.39, 0.48, 0.52, and 0.56) and two (0.52 and 0.56) were detected respectively on silicagel thin layer chromato-

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grams (TLC) using diazotized benzidine or ferric chloride as spraying reagent, and toluene : formic acid : ethyl formate (5:1:4, v/v) as developer. Among the components described above, compound I (R_f 0.52) and compound II (R_f 0.56) were successfully isolated by silicic acid column chromatography using the solvent system of chloroform : acetone. Yield of the compound I was 0.07 % and that of the compound II was 0.23 % against dried wood meal. Each compound was identified as described below.

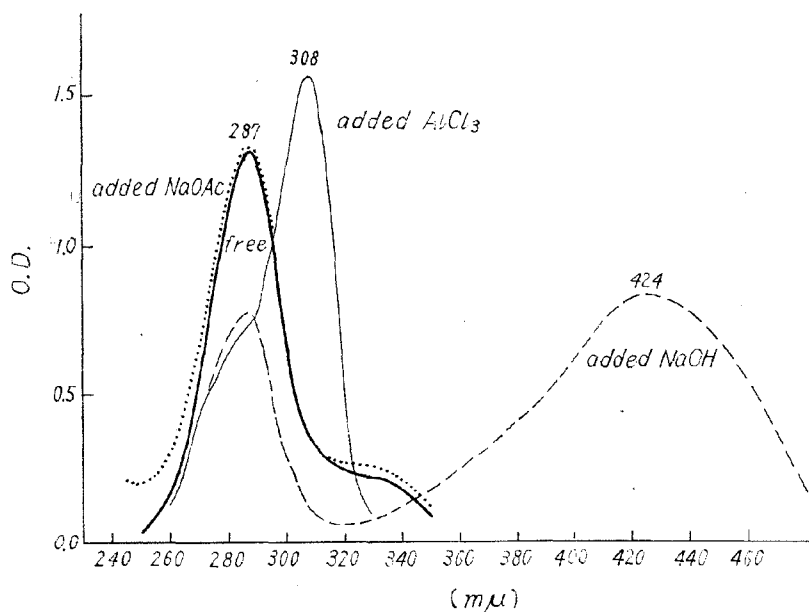


Fig. 2. UV Absorption Curves of Sakuranetin.

The result of magnesium—hydrochloric acid test for flavonoid compounds was positive, and showed crimson coloration as is the case with flavanones. The compound II also showed other color reactions; yellow for conc. sulfuric acid, hydrochloric acid or sodium hydroxide, purple brown for 1 % ferric chloride, pink for 1 % aluminium chloride (also a blue fluorescence under ultraviolet light), and violet to brown with conc. nitric acid. Among the alkaline (potassium hydroxide) degradation products, *p*-hydroxybenzoic acid was identified with using TLC-chromatogram. Therefore, this compound is considered to be a kind of flavanone bearing one hydroxyl group at position 4'.

Compound II, white needles, melted at 74–75°C, and its elementary analysis required a monohydrate, $C_{16}H_{14}O_5 \cdot H_2O$. Molecular weight was 297 (Theor. 304) in acetone. Methoxyl content was 9.84 % (Theor. 10.2 %).

Diacetate, chalcone acetate and monomethyl ether of the flavanone showed reasonable results with those from sakuranetin, in melting points and IR spectra, respectively. These data and others given below agreed well with previous results^{2), 5)} and were in support of identifying this compound as sakuranetin (4', 5-dihydroxy-7-methoxyflava-

none).

The compound I (Rf 0.52), yellow needles, melted at 164–165°C, elementary analysis required a molecular formula, $C_{16}H_{14}O_5$. Molecular weight was 288 (Theor. 286). Magnesium—hydrochloric acid test was negative. The chalcone which was derived from synthesized sakuranetin according to the Puri and Seshadri's procedure⁶⁾ from the isolated flavanone coincided with this yellow compound. It is not decided whether this chalcone is an artefact produced during isolation.

TLC data could not distinguish the flavanone isolated above from naringenin-7-monomethyl ether which was synthesized from naringenin (II). The diazomethane treated flavanone and naringenin dimethyl ether were identical on the TLC chromatogram.

In the ultraviolet absorption spectrum of this flavanone in ethanol, a typical peak for flavanones appeared as shown in Figure 2. The $\lambda_{\max}$ was 287 m μ ($\epsilon=18,720$, literature showed 287 m μ in sakuranetin). Bathochromic shift of peaks due to adding of sodium acetate, aluminium chloride or sodium hydroxide were 0, 21 and 137 m μ in each case. It was confirmed that hydroxyl groups were present at positions 5 and 4' of the flavonoid skeleton, but not at 7.

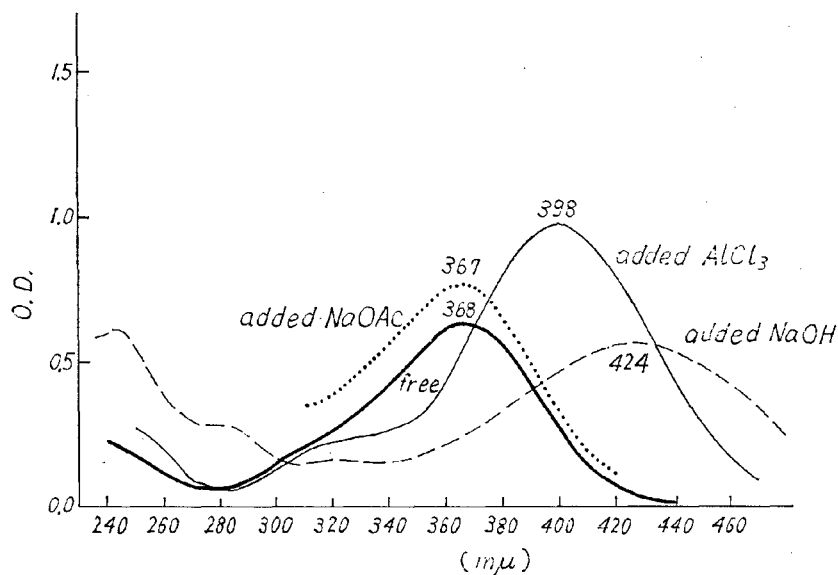


Fig. 3. UV Absorption Curves of Sakuranetin chalcone.

The yellow compound showed one broad peak suggesting a chalcone at $\lambda_{\max}$ 368 m μ ($\epsilon=27,880$), and this peak was shifted little by adding of sodium acetate, but bathochromically shifted by 30 m μ with adding of aluminium chloride, and 56 m μ with adding of sodium hydroxide. This result is in accordance with the 4, 2', 6'-trihydroxy-4'-methoxy-chalcone ($\lambda_{\max}$ 370 m μ , given in Horowitz and Jurd's literature⁷⁾).

IR absorption spectra of the isolated flavanone ($\nu_{\text{C=O}}$ 1650 cm^{-1}) and synthesized sakuranetin and also their acetates agreed satisfactorily with each other. The shift of

carbonyl absorption from synthesized flavanone (1715 cm^{-1} , Fig. 4) could be accounted for the effect of hydrogen bonding between hydroxyl group at 5 and carbonyl group at 4 and the effect of methoxyl group at 7.

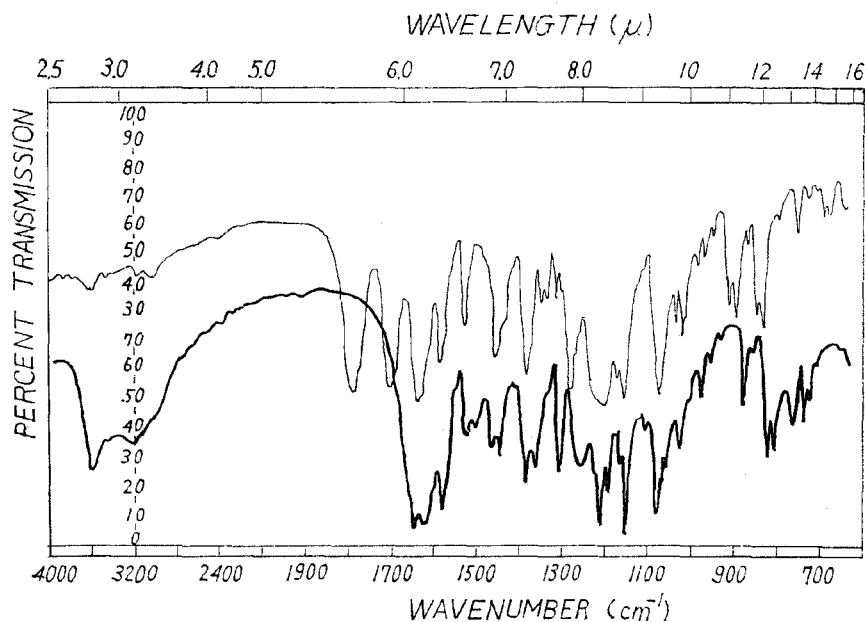


Fig. 4. IR Spectra of sakuranetin (lower) and sakuranetin diacetate (upper).

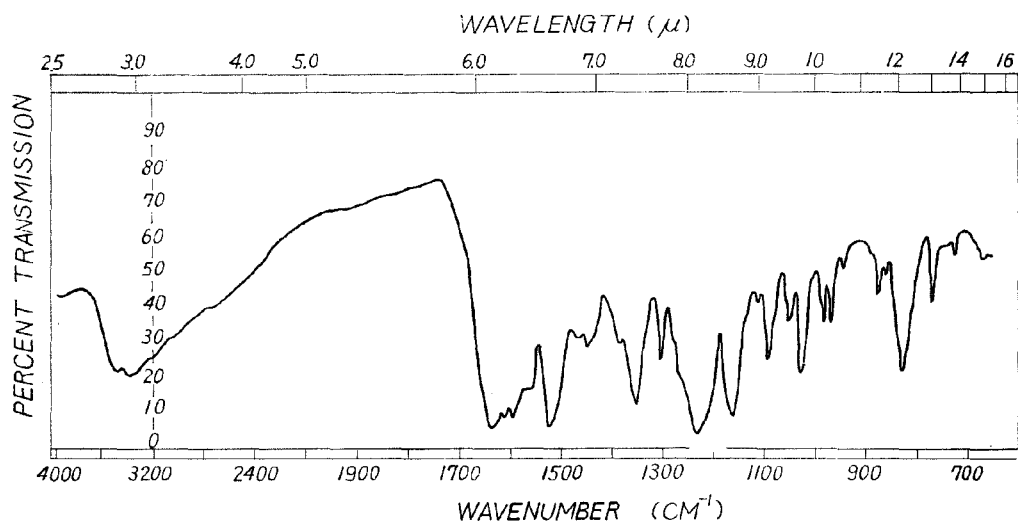


Fig. 5. IR Spectrum of sakuranetin chalcone.

IR spectra of the chalcone obtained from the wood and the synthesized sakuranetin chalcone also coincided with each other. (Fig. 5)

Further, NMR spectrum of the flavanone could be interpreted satisfactorily and the assignments, in which Batterham et al.⁹⁾ and Grouiller's papers¹⁰⁾ were useful, for peaks are given in Table 1.

Table 1. Nuclear magnetic resonance data of sakuranetin.

Absorption (τ)	J (c/s)	Relative intensity	Assignment
-2.00 (singlet)		1H	C-5 (-OH)
2.67 (doublet)	8	2H	C-2', -6'
3.13 (")	8	2H	C-3', -5'
3.92 (singlet)		2H	C-6, -8
4.64 (quartet)		1H	C-2
4.50 (singlet)		1H	C-4' (-OH)
6.20 (")		3H	C-7 (-OCH ₃)
6.90 (")		1H	C-3 trans
7.12 (doublet)		1H	C-3 cis
8.30 (singlet)		2H	water

Tetramethylsilane was added as internal standard. Sample was dissolved in warm deuteriochloroform.

Experimental

Apparatus

Unless otherwise stated, melting points were determined with the Yanagimoto micro-melting point apparatus using a microscope and results were not corrected. Ultraviolet absorption spectra were measured with the Shimadzu QB-50, infrared absorption spectra (KBr disc) with the Jasco IR-S, NMR spectra with the Varian A-60 at 60 Mc/sec. Molecular weight was determined with the Mechrolab vapor pressure osmometer 302. Thin layer chromatograms were carried out using Silicagel G (Merck).

Preparation of phenolic fraction

Finely ground heartwood (1040 g) was extracted with ether using large sized Soxhlet's extractor fitted with 3 l receiver for 12 hours until yellow color of the extract disappeared. After evaporating under reduced pressure, 58 g (5.6 % on wood meal) of viscous brown material was obtained. The material obtained was treated with aqueous 5 % sodium bicarbonate, 5 % sodium carbonate, and 5 % sodium hydroxide successively to give fractions 3.1 g (5.4 %), 27.0 g (47.1 %) and 18.7 g (32.6 %) respectively. Neutral fraction 3.1 g (5.4 %) (residue from above alkaline treatment) and ether-insoluble substance 5.5 g (9.6 %) produced during alkaline treatment were not investigated further.

Column chromatography

A glass column (5 cm×50 cm) was filled with 400 g of silicagel (0.2-0.5 mm) with chloroform, and 10 g of the sodium hydroxide soluble fraction, dissolved in a small amount of ether, was transferred on the top of the silicagel layer. The column was eluted successively with chloroform, chloroform-acetone (80:20, v/v, and 50:50, v/v), acetone and methanol. White needles from condensed eluate from chloroform, yellow needles from condensed eluate from chloroform-acetone (80:20 v/v) were obtained.

Both were recrystallized twice from boiling 60 % aqueous ethanol. The yields were 2.43 g (0.23 %) for white needles (double melting points, 75° and 140°) and 0.78 g (0.075 %) for yellow needles (m. p. 164-165°).

Sakuranetin

The white needles melted after repeated purification at 74-75° and 139-140°C.

Found: C, 63.17 %; H, 5.32 %

Calcd. for $C_{16}H_{14}O_5 \cdot H_2O$: C, 63.17 %; H, 5.31 %

Molecular weight determined in acetone was 297 (Theor. 304). Methoxyl content was 9.84 % (Theor. 10.3 %). Ultraviolet absorption maximum in absolute ethanol appeared at 287 m μ ($\epsilon=18,720$).

Preparation of sakuranetin from naringenin¹¹⁾

To 200 mg of naringenin (commercial product, containing a small amount of impurities which was detected chromatographically) in 10 ml of ether, was added one ml. of diazomethane-ether solution, then after adding a few drops of methanol, kept at room temperature for a short time. Sakuranetin was isolated from the mixture of the products by column chromatography with 20 g of silicagel and elution with chloroform. On recrystallization from aqueous ethanol it melted at 67° (96 mg). IR spectrum and R_f value of TLC coincided with the natural flavanone obtained above.

Sakuranetin monomethyl ether

Two hundred mg of the flavanone was dissolved in 5 ml of methanol and added 5 ml of ether solution of diazomethane (5 ml). After keeping in a refrigerator overnight, the solvent was removed and the residue was recrystallized from methanol. White crystal melted at 110°C (liter. 117°C),¹¹⁾ and yield was 100 mg.

Sakuranetin acetate

Three ml of acetic anhydride was added to 500 mg of sakuranetin, and further a drop of sulfuric acid, after a while the reaction mixture was poured into 100 ml of water. Crude products were extracted from the aqueous solution with ether and crystallized from a few ml of ethanol to give acetate melting at 96-97°C (liter. 97°).²⁾

Another acetylation product of sakuranetin was obtained by refluxing sakuranetin in acetic anhydride with sodium acetate. This acetylation product melted at 141-142° (liter. 132°).¹¹⁾

Found: C, 63.69 %; H, 4.99 %

Calcd. for $C_{22}H_{20}O_8$, C, 64.10 %; H, 4.89 %

This product was essentially an acetate of sakuranetin chalcone which was produced by the cleavage of the dihydropyrone ring of sakuranetin followed by the introduction of three acetyl groups.

Sakuranetin oxime

Five hundred mg of sakuranetin, 300 mg of hydroxylamine hydrochloride and 200

mg of sodium acetate were dissolved in 5 ml of ethanol and refluxed for 2 hours. After removing ethanol, the residue was dissolved in 10 ml of water and filtered. Pale yellow needles were obtained after recrystallization from hot 50 % ethanol. The yield was 520 mg which melted at 88°C (dehydration) and 190–192°C (liter. 195°).²⁾

Found: C, 63.54 %; H, 5.06 %; N, 4.62 %

Calcd. for $C_{16}H_{15}O_5N$: C, 63.78 %; H, 5.02 %; N, 4.65 %

Preparation of Sakuranetin chalcone

Fifty mg of the flavanone was dissolved in 10 ml of 5 % aqueous sodium hydroxide solution and heated on a hot plate at 60°C for one hour. Color of the solution changed into deep orange from yellow. After cooling, the reaction mixture was neutralized with 5 % citric acid, and extracted with ether. TLC chromatogram of the reaction product revealed the presence of unchanged sakuranetin and the yellow chalcone in nearly equal amounts. R_f value of the latter coincided with that of chalcone obtained from the wood.

Alkaline degradation

Two g of potassium hydroxide, 4 ml of water and 120 mg of the flavanone was heated in a 50 ml nickel crucible for one hour at 200°C. After cooling, solid material in the crucible was crushed with a glass rod and dissolved in 30 ml of water. Filtrate through a glass filter was acidified with 2N-sulfuric acid and extracted with ether. Sixty mg of the ether soluble material was obtained, and a small portion of this material was applied to TLC for identifying phenols in the degradation products. TLC chromatogram of silicagel G was developed with the solvent system of toluene: formic acid: ethyl formate (5:1:4, v/v) and sprayed with 2 % ferric chloride solution. Yellow spot of *p*-hydroxybenzoic acid was clearly identified among several spots at R_f value 0.50.

Sakuranetin chalcone

The yellow needles isolated from wood melted at 164–165°C. Ultraviolet absorption maximum appeared at 368 m μ (ϵ =27,880) in absolute ethanol. Molecular weight in acetone was 288 (Theor. 286).

Found: C, 66.77 %; H, 4.98 %

Calcd. for $C_{16}H_{14}O_5$: C, 67.20 %; H, 4.93 %

The acetate prepared with boiling acetic anhydride and sodium acetate melted at 142–144°C.

Found: C, 63.65 %; H, 4.95 %

Calcd. for $C_{22}H_{20}O_8$: C, 64.20 %; H, 4.89 %

Acknowledgement

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giving us the wood, and we also thank Prof. Dr. T. Mitsui, Dept. of Agricultural Chemistry, Kyoto University, for elementary analysis, Dr. T. Shingu, Faculty of Pharmacology, Kyoto University, for NMR spectra and Dr. S. Sudo, Forest Experimental Station, Ministry of Agriculture, Tokyo, for identifying of wood species.

Summary

Sakuranetin (4', 5-dihydroxy-7-methoxyflavanone) has been isolated from the ether extract of the wood of *Haplormosia monophylla* HARMS (family *Leguminosae*, subfamily *Papilionatae*), an African wood, in a yield of 0.23 %. It seems to be the first case that the compound was found in the wood of *Leguminosae* other than *Prunus* sp. (bark and wood), *Eucalyptus* sp. (kino) or *Juglans* sp. (bark). The above fact is much interesting in the view of chemo-taxonomy. The isolated compound was confirmed chemically also with the aid of UV, IR, and NMR spectra. Another compound, sakuranetin chalcone has been also isolated and confirmed from the same wood, but this compound might be an artefact produced during isolating process.

摘 要

ガボン国に産する有用材, イデワ (IDEWA) 学名 *Haplormosia monophylla* HARMS の材成分について検索した結果, そのエーテル可溶成分から 0.24 % の収量で白色結晶を得ることができ, さらにこのものと誘導体について化学分析を機器分析とともに行なった結果, サクラネチン (=4', 5-dihydroxy-7-methoxyflavanone) であることを確かめた。従来, この化合物は数種のサクラ (*Prunus* sp.) の樹皮, 材に発見されており, 他にはユーカリ (*Eucalyptus* sp.) のキノ (kino) とテウチグルミ (*Juglans* sp.) の樹皮にその存在を認められている。マメ科の材に見付けられた例として, 化学的植物分類学の立場より非常に興味深いことであつた。

なお, 同時に黄色結晶も単離し, サクラネチンシャルコンであることを確かめたが, この場合は抽出操作中の変成物である可能性をも含んでいる。

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