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Structures of New Flavonoids, Sophoradochromene and Sophoranochromene, from *Sophora subprostrata*

In the previous paper,[1] we reported the structures of sophoradin (I) and sophoranone (II) which were isolated from the root of *Sophora subprostrata* CHUN et T. CHEN (Chinese Drug: Shan-Dou-Gen (山豆根)).

In our further studying on the constituents of this drug, two new flavonoids were isolated.

The present communication deals with the structures of these flavonoids, for which we now give the names, sophoradochromene (III) and sophoranochromene (VII), respectively.

Sophoradochromene (III) was obtained as yellow needles; mp 154°; $C_{30}H_{34}O_4$; $FeCl_3$ (+); UV λ_{max}^{EtOH} mμ (log ε): 380 (4.50); $\lambda_{max}^{EtOH\text{-}AlCl_3}$ mμ (log ε): 435 (4.57); $\lambda_{max}^{EtOH\text{-}NaOEt}$ mμ (log ε): 420 (4.50); IR (KBr) cm^{-1}: 3210 (OH), 1625 (conjugated CO), 1600, 1590 and 1550 (aromatic C=C), 1380 ($-CH_3$).

The UV spectrum suggested the presence of chalcone nucleus in III,[2a] which was also supported by the formation of a dihydrochalcone derivative (octahydrosophoradochromene) (IV), mp 134°, $C_{30}H_{42}O_4$; UV λ_{max}^{EtOH} mμ (log ε): 289 (4.20); IR (KBr) cm^{-1}: 3200 and 3150 (OH), 1615 (conjugated CO), 1600 (aromatic C=C), 1385 and 1370 ($-CH_3$), by catalytic hydrogenation. On acetylation, IV gave a diacetate (V)[3]; $FeCl_3$ (—); NMR[4]: 7.71 (3H, s., -OAc), 7.75 (3H, s., -OAc).

In the NMR spectrum of III, two sharp singlets at −3.9 (1H) and 3.8 (1H, shifted to 3.91 at 50°) showed the presence of two hydroxyl groups. Two vinyl doublets (J=9.7 cps) at 3.72 (1H) and 4.38 (1H) in conjunction with a singlet at 8.56 (6H) for two methyl groups suggested a 2,2-dimethylchromene ring. Two singlets at 8.25 (6H) and 8.18 (6H) for four olefinic methyl groups and a broad triplet at 4.72 (2H) due to vinylic protons split by adjacent methylene groups, associated with a pair of doublets (J=7.5 cps) at 6.55 (2H) and 6.75 (2H) suggested the signals characteristic of two γ,γ-dimethylallyl groups. Furthermore, these signals in III disappeared in IV, while a sharp singlet at 8.7 (6H, $\underset{-O}{C}\!>\!C\!<\!\underset{CH_3}{\overset{CH_3}{}}$), two sharp doublets ($J$=6 cps) at 9.05 and 9.07 (total intensity of 12H, $-CH\!<\!\underset{CH_3}{\overset{CH_3}{}}\times 2$) and a broad multiplet at 7.0—8.5 due to methylene groups appeared in IV.

Alkali fission of III with 50% KOH gave a degradation product, mp 160°, $C_{13}H_{16}O_3$, which was a fragment corresponding to A-ring and proved to be identical with VI by comparison with authentic sample from sophoradin (I).[1]

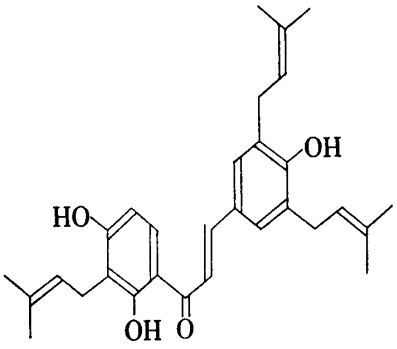
In the NMR spectrum of III, coupled doublets centred at 2.25, 2.65 (J=15 cps) and 2.79, 2.91 (J=2 cps) represent two pair of protons which are assigned to olefinic protons of the chalcone nucleus (C-β,α-H) and *meta* proton pair of the B-ring, respectively.

These data indicated that the remaining a isopentenyl group and a 2,2-dimethylchromene ring must be placed at the B-ring, and at the same time, which led to the four possible substitution patterns (IIIa, b) (IIIc, d) for this ring.

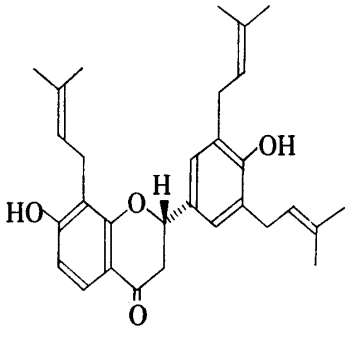
The partial formula IIIa is presumed to be preferred on considering the co-existence of III with I. The decision was made by the transformation of I to III. Refluxing I

1) M. Komatsu, T. Tomimori, K. Hatayama, Y. Makiguchi, and N. Mikuriya, *Chem. Pharm. Bull.* (Tokyo), **17**, 1299 (1969).
2) *a*) L. Jurd, "The Chemistry of Flavonoid Compounds," T.A. Geissman, ed., Pergamon Press, London, 1962, pp. 141—147; *b*) W.B. Whalley, *ibid.*, pp. 441—467.
3) The product was failed to be crystallized, but its purity was certified by thin-layer chromatography.
4) All NMR spectra were taken at 60 Mcps in $CDCl_3$ with TMS as an internal standard. Chemical shifts were given in τ values. Abbreviations: s.; singlet, d.; doublet, t.; triplet, m.; multiplet, br.; broad.

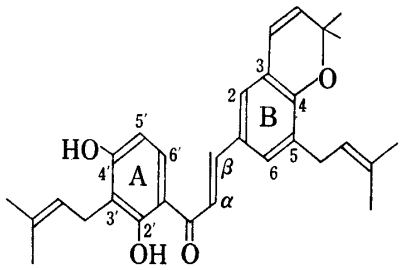
in pyridine containing some piperidine afforded (±)-sophoranone[1] and a small amounts of III.



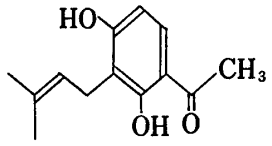
I



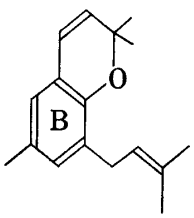
II



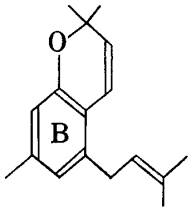
III



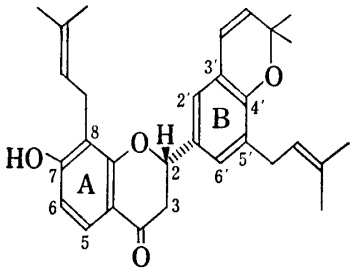
VI



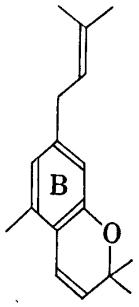
IIIa



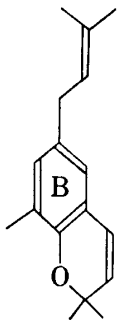
IIIb



VII



IIIc



IIId

Thus, sophoradochromene could be formulated as III.

Sophoranochromene (VII) was obtained as colorless needles, mp 152°, $C_{30}H_{34}O_4$. Physical properties: $[\alpha]_D^{25}$ −63.9 (c=0.57 in EtOH); IR (KBr) cm^{-1}: 3270 (OH), 1660 (conjugated CO), 1600 and 1590 (aromatic C=C), 1385 and 1370 (-CH_3); UV λ_{max}^{EtOH} mμ (log ε): 286 (4.20); $\lambda_{max}^{EtOH\text{-}NaOH}$ mμ (log ε): 262 (4.22),348 (4.49); NMR: 8.56 (6H, s., $-O>C<\underline{CH_3}\ \underline{CH_3}$), 8.29 (12H, s., $C=C<\underline{CH_3}\ \underline{CH_3}\times 2$), 7.1 (2H, m., C-3-$H_2$), 6.7 (4H, double d., J=7.5 cps, Ar-$C\underline{H_2}$-CH=C<×2), 4.6—4.8 (3H, m., C-2-H and -CH_2-$C\underline{H}$=C<×2), 4.41 and 3.72 (2H, d., J=9.7 cps, -$C\underline{H}$=$C\underline{H}$-), 3.46 (1H, d., J=8 cps, C-6-H), 3.12 and 2.97 (2H, d., J=2 cps, C-2′, 6′-H), 2.5 (1H, s., C-7-O$\underline{H}$, shifted to 2.87 at 50°), 2.30 (1H, d., J=8 cps, C-5-H).

VII was suggested a flavanone corresponding to III from its spectral characteristics and the formation of following derivatives. VII gave a hexahydro derivative, mp 212°, $C_{30}H_{40}O_4$ (VIII), by catalytic hydrogenation, and VIII further formed a monoacetate, mp 92°, $C_{32}H_{42}O_5$, by acetylation.

VII was readily cleaved to III by a short treatment with hot 10% NaOH. Furthermore, refluxing III in pyridine–piperidine regenerated (±)-sophoranochromene (VII′), mp 152°, which exhibited no optical rotation and did not depress the melting point on admixture with natural sophoranochromene (VII). The spectra (IR, UV and NMR) of VII′ were also found to be superimposable with those of VII.

Since all natural (−)-flavanones generally have S-chirality at C-2,[2b] the structure VII could be given to sophoranochromene.

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Microdetermination of Glucose using 3-Methyl-2-benzothiazolone Hydrazone Hydrochloride and Glucose Oxidase-Catalase Enzyme System

Glucose oxidase–catalase enzyme system has recently been introduced into the assay of glucose in biological fluids.[1,2] This system is claimed to be more sensitive and reproducible than glucose oxidase-peroxidase system when chromotropic acid–sulfuric acid reagent is used for color development.[1] However, this method requires heating the sample with concentrated sulfuric acid which may react with other substances than glucose to give color and, in addition, the concentrated acid may be troublesome to handle in a clinical laboratory. The method described in this paper utilizes 3-methyl-2-benzothiazolone hydrazone hydrochloride (MBTH), a highly sensitive reagent for aliphatic aldehydes,[3] in place of chromotropic acid–sulfuric acid reagent. MBTH was found to react with formaldehyde generated by the action of catalase on methanol at pH 5.6 which is at the same time optimal hydrogen ion concentration for glucose oxidase. This method also proved to be more sensitive and simple than the chromotropic acid method.

Reagents——1) Enzyme Reagent: 0.4 g of glucose oxidase (GOD-III, Boeringer, Mannheim) is dissolved in 20% methanol to make 100 ml. 2) Phosphate buffer, pH 5.6. 3) 0.5% MBTH aqueous solution. 4) Ferric Chloride Reagent: 0.83 g of $FeCl_3.6H_2O$ is dissolved in 10% $KHSO_4$ aqueous solution to make 100 ml.

Procedure——To 1 ml of sample solution containing 1 to 20 μg of glucose is added 1 ml of enzyme reagent and 2 ml of phosphate buffer and the mixture is incubated at 37°—38° for 90 min. Then 0.5% MBTH is added and the resulting mixture is allowed to stand at room temperature for 60 min. Finally, ferric chloride reagent is added and the absorbance is read after 60 min at 620 mμ against the reagent blank. Linear relationship is observed in the range of 1 μg to 20 μg per ml of sample solution.

When applied to human serum, to which is added 100 mg and 200 mg of glucose per ml, 100.0 and 102.5% recovery was observed, respectively. Somogyi protein precipitation meth-

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2) S. Ikawa and T. Obara, *Jap. J. Clin. Path.*, **13**, 197 (1965).
3) E. Sawicki, T.R. Hauser, T.M. Stanley and W. Elbert, *Anal. Chem.*, **33**, 93 (1961); This reagent was recently applied by the authors to microdetermination of amino sugars: A. Tsuji, T. Kinoshita, and M. Hoshino, *Chem. Pharm. Bull.* (Tokyo), **17**, 217 (1969); *idem*, *ibid.*, in press.