Tetrahedron Letters No.15, pp. 1611-1617, 1966. Pergamon Press Ltd.
Printed in Great Britain.

STUDIES ON THE CONSTITUENTS OF SWERTIA JAPONICA: ISOLATION AND STRUCTURE OF NEW FLAVONOIDS, SWERTISIN AND SWERTIAJAPONIN.

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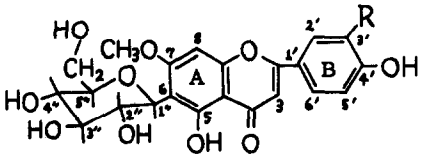
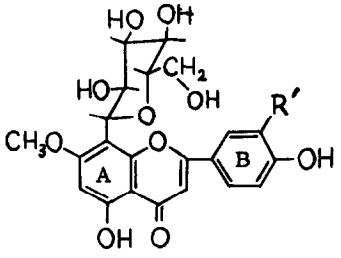
(Received 4 February 1966)

Swertisin was first isolated from the whole herb of Swertia japonica (Fam: Gentianaceae) by Nakaoki (1), who proposed the empirical formula $C_{13}H_{10}O_6 \cdot H_2O$. Subsequently, Asahina et al. revised the formula for swertisin to $C_{23}H_{24}O_{11}$(2). No further investigations, however, have been made.

We now found that crude swertisin, obtained from this plant by Nakaoki's procedure, consisted of swertisin and a small amount of two other flavonoid compounds, which were isolated in pure state on polyamide chromatography i.e. compound (I), m.p. 243° (decomp.) : compound (II), m.p. 265° (decomp.) and compound (III), m.p. 237° (decomp.). Compound (II) was a new flavonoid, which would be named swertiajaponin hereafter, and compound (III) was identical with homo-orientin (m.p. and mixed m.p. 237°, I.R., and U.V. spectra identical with an authentic sample).

The present paper deals with the elucidation of the structures of swertisin (I) and swertiajaponin (II), which have now been established as I and II respectively.





(I) R = H
(II) R = OH
(I') R'= H
(II') R'=OH

Compound (I) (I), identified with authentic swertisin, was obtained in pale yellow needles, m.p. 243° (decomp.), and its analytical values suggested the formula $C_{22}H_{22}O_{10}$ containing one methoxyl group. It gave a greenish brown color with ferric chloride, and the reduction tests for flavonoid (Mg-HCl and Zn-HCl) were positive.

U.V. absorption: $\lambda_{max.}^{EtOH}$ 273 mμ and 336 mμ (log ε, 4.24 and 4.32 respectively); $[\alpha]_D^{20}$ - 10.0 (c, 0.9, pyridine). It gave a hexa-O-acetate, m.p. 155° - 158°, $C_{22}H_{16}O_{10}(COCH_3)_6$, on boiling it with acetic anhydride in the presence of pyridine, which gave negative ferric reaction indicating the existence of six hydroxyl groups. Methylation of I with diazomethane yielded di-O-methylswertisin (IV), m.p. 302°, $C_{21}H_{17}O_7(OCH_3)_3$, which gave no coloration with ferric chloride, and no significant bathochromic shift of U.V. absorption maxima by adding alminum chloride. The methylether (IV) further formed its tetra-acetate, m.p. 150°-155°, or tetra-p-nitrobenzoate, m.p. 236°, indicating that out of the six hydroxyls in I, two are phenolic and the rest alcoholic.

Alkali fission of I afforded phloroglucin monomethylether, p-hydroxybenzoic acid and p-hydroxyacetophenone. Boiling it with hydriodic acid in phenol gave apigenin which was further characterized as its

triacetate. Oxidation of IV with nitric acid yielded p-anisic acid. The presence of a 7-methoxyl group in I was confirmed by comparing its U.V. spectrum with those in the presence of sodium acetate (no change) and of alminum chloride (bathochromic shift of 44 mμ) (3). These results indicated that swertisin contained a genkwanin unit, with a - $C_6H_7(OH)_4$ moiety attached to the nucleus.

On the other hand, swertisin was also found to be non-glycosidic because of its negative Molisch reaction and non-formation of sugar even after drastic treatment with conc. hydrochloric acid, and so it was suggested that swertisin might be a C-glycosyl compound. The estimation of periodate consumed for the oxidation of di-O-methyl-swertisin resulted in an uptake of two moles of the oxidant within five hours with the formation of one mole of formic acid. On ozonolysis of swertisin, D-glucose and D-arabinose were produced which were determined by paper chromatography. These results show that the C_6-moiety in swertisin is D-glucose in the form of a pyranose. Further, hydrolytic decomposition of di-O-methylswertisin with boiling aqueous barium hydroxide gave p-methoxyacetophenone and a degradation product, $C_{14}H_{16}O_7(OCH_3)_2$ (V), which was a fragment corresponding to A-ring and formed penta-acetate, m.p. 169°-170°, $C_{14}H_{11}O_7(OCH_3)_2(COCH_3)_5$. With the excess of aqueous periodic acid followed by reduction, V gave rise to 2 : 4-dimethoxy-3-methyl-6-hydroxyacetophenone (2 : 4 - dinitrophenylhydrazone, m.p. 205°) which was identified by comparing it with a synthetic specimen starting from phloroglucinol (4, 5). From these results, it is evident that the

glucopyranosyl residue in swertisin is present in the 6-position of the genkwanin mucleus. This conclusion is also supported by the fact that swertisin gives a positive Gibbs indophenol test. Moreover, it is supported by n.m.r. studies that the glucopyranosyl residue must have β-configuration.

In the spectrum* of hexa-O-acetylswertisin (34H), four proton signals display typical signal patterns for B-ring protons (AB type) : $H_{2',6'}$, doublet (J=9.c.p.s.) at τ 2.25 ; $H_{3',5'}$, doublet (J=9.c.p.s.) at τ 2.85. Two singlets at τ 3.23 (1H) and τ 3.55 (1H) could be assigned to the C_8 and C_3 proton respectively. A signal at τ 6.05 (3H) indicated the presence of one methoxyl group. A total of 18 protons is observed over the range τ 7.57 - 8.25, and these are attributable to the six acetyl groups. The signals over the range τ 4.0 - 6.0 account for the seven protons of the glucosyl residue. One of these, a doublet centred at 5.10 is assigned to the $C_{1''}$ proton, the large coupling constant (J=10 c.p.s.) due to a trans-diaxial coupling with the $C_{2''}$ proton indicating a β-C-glucopyranosyl residue.

Consequently, the structure of swertisin was established as 6-C-β-D-glucopyranosyl genkwanin.

Compound (II), named swertiajaponin, gave the following color reactions, i.e. $FeCl_3$(+), Mg-HCl(+), Zn-HCl(+), Gibbs(+), and Molisch (-). U.V. absorption : $\lambda_{max.}^{EtOH}$ 259 mμ (shoulder), 271 mμ and 350 mμ (log ε , 4.30, 4.31 and 4.38 respectively) ; $[\alpha]_D^{20}$ -2.6° (c, 0.5,

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* All n.m.r. spectra were recorded on a JNMC-60 spectrometer (Japan Electron Optics Laboratory Co., Ltd.), using $CDCl_3$ as solvent with T.M.S. as internal standard.

pyridine). Its molecular formula worked out to $C_{22}H_{22}O_{11}\cdot 1/2\ H_2O$ containing one methoxyl group. It formed a hepta-acetate, $C_{21}H_{12}O_{10}$ (OCH_3) $(COCH_3)_7$, whose I.R. spectrum showed the absence of hydroxyl group. Methylation with diazomethane gave tetra-O-methylhomo-orientin, $C_{21}H_{16}O_7(OCH_3)_4$, identified with authentic sample by m.p., mixed m.p., co-chromatography on paper, and I.R. spectra, showing that swertiajopinin might be homo-orientin-monomethylether. The presence of a 7-methoxyl group in II was confirmed by the comparison of its U.V. spectrum with those in the presence of sodium acetate (no change) and of alminum chloride (bathochromic shift of 80mμ), and the presence of a o-dihydroxy group was detected by the 28 mμ bathochromic shift of the long-wave length band on the addition of a mixture of boric acid and sodium acetate (3). These facts are also supported by the formation of following degradation products. Alkali fission of II gave phloroglucin monomethylether and protocatechuic acid. Boiling it with hydriodic acid in phenol gave luteolin.

Barium hydroxide fission of tri-O-methylswertiajoponin afforded 3 : 4-dimethoxyacetophenone and V (penta-acetate : m.p. and mixed m.p. 169° - 170°), which was proved to be identical with 3-C-β-D-glucopyranosyl-2 : 4-dimethoxy-6-hydroxyacetophenone by the n.m.r. spectrum of its penta-acetate, i.e. four acetyls of glucopyranosyl group at τ 7.93 (6H), 7.96 (3H) and τ 8.20 (3H) ; two methoxyls at τ 6.15 (3H) and 6.22 (3H) ; Ar-O Ac at τ 7.75 (3H) ; Ar-Ac at τ 7.48 (3H) ; benzenoid proton at τ 3.57 (1H) ; and the rest of seven protons at τ 4.0 - 6.0 indicated same signal patterns as those of β-glucopyranosyl residue on

hexa-O-acetylswertisin.

From these results, the structure of swertiajaponin was established as 6-c-β -D -glucopyranosylluteolin-7-methylether.

The n.m.r. spectrum of hepta-O-acetylswertiajaponin is as follows. Three proton signals could be assigned to B-ring protons : $H_{2'}$ doublet (J=2 c.p.s.) at τ 2.26 ; $H_{5'}$, doublet (J=8 c.p.s.) at τ 2.63 ; and $H_{6'}$, poorly resolved quartet (J_{meta}= 2 c.p.s., J_{ortho}=8 c.p.s.) at τ 2.25. Two singlets at τ 3.11 (1H) and τ 3.45 (1H) could be assigned to the C_8 and C_3 proton respectively. A singlet at τ 6.00 (3H) indicated one methoxyl, and a total of 21 protons, observed over the range τ 7.56-8.21,are attributable to the seven acetyl groups. The signals over the range τ 4.0 -6.0 (7H) displayed same signal patterns as those of hexa-O-acetylswertisin.

In addition, parallel treatment of swertisin and swertiajaponin under the usual hydrolytic conditions yielded equilibrium mixtures both of which produced two spots on paper *, ones being identical in Rf-values with the original swertisin and swertiajaponin, and the others, giving the lower Rf. values, which were suggested to be the 8-C-isomers due to a Wessely-Moser rearrangement, considering the interconvertibilities between vitexin and isovitexin (6,7), or orientin and homo-orientin (8,9). The isomers, therefore, would be named isoswertisin (I') and isoswertiajaponin (II') respectively.

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* Paper chromatography was carried out, using solvent systems, of (1) BuOH-AcOH-H_2O (4 : 1 : 5), (2) 60% AcOH, and (3) 15% AcOH.

Acknowledgement, The samples of swertisin and luteolin, apigemin and homo-orientin, and vitexin and orientin were kindly supplied by Prof. N. Morita in Toyama University, Prof. M. Yasue in Nagoya City University, and Dr. M. Aritomi in Kumamoto University respectively, to whom the author's thanks are due.

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