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A NEW TYPE OF SAPONIN FROM STYRAX OFFICINALIS L.

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The pericarps of Styrax officinalis L., an East mediterranean plant, have been used by local fishermen as fish poison. Since a saponin (jegosaponin) has been isolated from Styrax japonica Sieb. et Zucc. (1,2), a botanically related plant, it appeared possible that Styrax saponins are responsible for the ichthyotoxic action. It was therefore decided to investigate the saponins of Styrax officinalis L.

By using a modification of Wall's method (3), we succeded in isolating an unknown saponin in pure crystalline form, of m.p. 242°, $[\alpha]_D$ in ethanol +33.3° (Found: C,56.5; H, 7.6. $C_{58}H_{88}O_{27} \cdot H_2O$ requires: C, 56.4; H, 7.3. $C_{58}H_{90}O_{27} \cdot H_2O$ requires: C, 56.3; H, 7.4).

The new compound is a very strong foaming agent; 0.5 µg/ml of this saponin produces 50% haemolysis of a 2% suspension of cattle erythrocytes. Since the H_{50} value * of

* H_{50} indicates the concentration producing 50% haemolysis.

digitonin, one of the most potent haemolytic agents, is 2.5 $\mu g/ml$, under the same conditions, the above figure testifies the extraordinary activity of the new saponin.

Acid hydrolysis of the new saponin yields a sapogenin, designated as "A", and a mixture of sugars. The chromatographic analysis of the mixture showed that the sapogenin molecule is bound to one mole of each of the following sugars: glucose, galactose, rhamnose and glucuronic acid. Sapogenin (A) has m.p. 257-8° and $[\alpha]_D$ in tetrahydrofuran +31° (Found: C, 71.1; H, 9.2; active hydrogen 1.03%. $C_{34}H_{48}O_6 \cdot H_2O$ requires: C, 71.6; H, 8.8; 6-active hydrogens 1.05%. $C_{34}H_{50}O_6 \cdot H_2O$ requires: C, 71.3; H, 9.1; 6-active hydrogens 1.05%).

By basic hydrolysis of sapogenin (A) a new sapogenin, designated as "B", was obtained together with benzoic acid. The saponification equivalent gives a minimum equivalent weight of 570 or 572 for sapogenin (A). Sapogenin (B) has the following properties: m.p. 312° (decomp.), $[\alpha]_D$ in tetrahydrofuran +26.6°. (Found: C, 72.3; H, 10.0; active hydrogen 1.05%. $C_{27}H_{44}O_5$ requires: C, 72.3; H, 9.8; 5-active hydrogens 1.12%. $C_{27}H_{46}O_5$ requires: C, 72.0; H, 10.2; 5-active hydrogens 1.11%).

Calculation of the expected analytical figures of the 3 substances reported assuming either C_{26} or C_{28} as basic carbon skeleton of sapogenin (B) shows disagreement with the values found.

Comparison of the analyses of sapogenin (A) and (B) shows that (A) is a benzoate of (B), the former containing in addition

a molecule of water. This water molecule can not be bound by covalent links because (A) has exactly one active hydrogen more than (B) (see above). If the water were covalently bound, the extra hydrogen of the hydroxyl group in sapogenin (A) would be balanced by the active hydrogen of the new alcoholic group in (B), liberated by ester hydrolysis.

As the Zerewitinoff analysis of sapogenin (B) shows, all 5 oxygens in this compound are present as hydroxyl groups. Moreover, sapogenin (B) yields di- and tri-acetates, having three and two active hydrogens respectively.

All known sapogenins with a C_{27} skeleton have a spiroketal side chain with the exception of kryptogenin (4). The latter however possesses only 4 oxygen atoms, two of which are present as carbonyl groups. The I.R. spectra of compounds (A) and (B) did not show any of the four absorption bands near 860, 900, 920, 980 cm^{-1}, characteristic for the spiroketal chain (5,6), nor did the U.V. spectra of these compounds in concentrated sulphuric acid show the peak near 270 mμ. This band again characterizes the spiroketal structure and is also obtained with kryptogenin (7).

The active principle of Styrax officinalis differs from the corresponding compound obtained from Styrax japonica, jego-sapogenin being a tiglic acid ester of a C_{30} sapogenol (2).

It appears therefore that the saponin obtained from Styrax officinalis L. represents a new type.

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