THE GLYCOSIDIC CONSTITUENTS OF *HYDROCOTYLE VULGARIS* L.

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From *Hydrocotyle vulgaris* L. the amorphous saponin earlier designated Hydrocotyle-saponin B has been isolated and its hydrolysis products studied.

A PRELIMINARY study of the saponins of *Hydrocotyle vulgaris* was reported in 1956[1]. The object was to ascertain whether the chemical components of this European plant were similar to those of a closely allied plant, *Centella asiatica* L., which occurs in Madagascar and Ceylon.

From the Madagascar variety the crystalline triterpene glycoside, asiaticoside, has been isolated[2]. Hydrolysis of this produced two molecules of glucose, two of rhamnose and one of a crystalline α-amyrin derivative asiatic acid ($C_{30}H_{48}O_5$)[3,4], which contains three hydroxyl groups and a carboxyl group.

The components of the Ceylonese variety were an amorphous triterpenoid glycoside, centelloside, as well as three amorphous polyhydroxy triterpenic acids, namely centic acid, centoic acid and centellic acid. On hydrolysis, centelloside split off glucose, fructose and centellic acid in the molecular ratio of 10 : 2 : 1[5,6]. None of these compounds appeared to be identical with asiatic acid or asiaticoside.

Previously[1] *H. vulgaris* in contrast to *C. asiatica*, was shown to possess haemolytic activity, equivalent to that of senega root. Using paper chromatography, four haemolytically active substances were detected which were named component S_4, hydrocotyle-saponin A_1 and A_2, and hydrocotyle-saponin B. Quantitatively this latter substance forms the most important haemolytically active component of the plant. Further work[7] was aimed at obtaining more information on the composition of hydrocotyle-saponin B isolated for this purpose from the dialysed methanol saponin prepared as described in earlier work[1].

EXPERIMENTAL

Purification of Saponin B

The dialysed methanol saponin[1] contained a small amount of coloured matter and saponin A_1, as impurity. This was removed by adsorption chromatography on slightly acid alumina and eluting saponin B with methanol containing 0·1–0·3 per cent w/v of formic acid. Saponin B was further purified by shaking a 1 per cent solution of the saponin in methanol (98 per cent v/v) with a little activated charcoal. After filtration and evaporation to dryness, the residue was taken up in 0·1N NaOH and electrodialysed to remove traces of $Al(OH)_3$ eluted from the column. The product was obtained as a white amorphous solid, free from ash, melting at 209–210° (decomp.). Hydrolysis, effected by heating in a

sealed tube at 100° for 17 hours a solution containing 2 per cent w/v of the saponin in equal volumes of methanol (98 per cent v/v) and 3 per cent sulphuric acid, gave products identified by paper chromatography as glucose, arabinose and glucuronic acid in about equal amounts.

From the products of hydrolysis the tentative formula $C_{47}H_{74}O_{20}$ or $C_{47}H_{76}O_{21}$ is suggested. After drying over P_2O_5 *in vacuo* for 72 hours (110° at 0·1 mm. Hg), when the moisture content was found to be 9·09 per cent, analysis gave the following results.

Found: C, 56·08; H, 7·98 per cent. $C_{47}H_{74}O_{20}$ requires C, 58·86; H, 7·78 per cent: $C_{47}H_{76}O_{21}$ requires C, 57·77; H, 7·84 per cent.

As saponin B retains solvent tenaciously, this may be responsible for the differences in the found and calculated percentages.

Hydrolysis Products

The aglycone obtained from hydrolysis was amorphous, and could be separated into three components by adsorption chromatography. The aglycone in solid state was first extracted with hot chloroform. This effected a partial separation of the components, which were named A-, I- and K-aglycone. Both the A- and I- and some K-aglycones were chloroform soluble. The chloroform insoluble fraction was entirely K-aglycone. The chloroform soluble fraction was evaporated to dryness and the residue taken up in a mixture of chloroform and ether (1 : 9 v/v). It was then chromatographed on slightly acid alumina, eluting the components with mixtures of chloroform-ether and chloroform-methanol in sequence of increasing polarity.

A-glycone. An amorphous haemolytically active substance of melting point 243–246° (decomp.). After drying over P_2O_5 *in vacuo* for 48 hours at 100°–0·1 mm. Hg, found: C, 72·0; H, 10·0 per cent. Calculated for $C_{30}H_{48}O_5$: C, 73·7; H, 9·9 per cent.

I-Aglycone. An amorphous haemolytically inactive substance of melting point 224–227° (decomp.). After drying over P_2O_5 *in vacuo* for 72 hours at 100° and 0·1 mm. Hg, found: C, 70·1; H, 9·6 per cent. Calculated for $C_{30}H_{50}O_6$: C, 71·1; H, 9·9 per cent. The differences between the found and calculated percentages are attributed to the tenacious manner in which the solvent is retained, an experience in common with Bhattacharrya[8], who was working with amorphous triterpenic acids from the Ceylonese variety of *C. asiatica.*

K-Aglycone. A crystalline haemolytically inactive substance of melting point 283° (decomp.). After drying over P_2O_5 *in vacuo* for 24 hours at 20° and 0·1 mm. Hg, found: C, 70·9; H, 10·1 per cent. Calculated for $C_{30}H_{50}O_6$: C, 71·1; H, 9·9 per cent.

Molecular weight estimated according to the method of Smit and others[9], found: 500 and 513, calculated: 506·7.

Acetates of K-aglycone. Penta-acetate (amorphous): after drying over P_2O_5 *in vacuo* for 48 hours at 60° and 0·1 mm. Hg, found: C, 67·0; H, 8·5; $COCH_3$, 29·0 per cent. Calculated for $C_{40}H_{60}O_{11}$: C, 67·0; H, 8·4; $COCH_3$, 30·0 per cent. Melting point: 223–226°.

Tri-acetate (crystalline): after drying over P_2O_5 *in vacuo* for 48 hours at 60° and 0·1 mm. Hg, found: C, 68·0; H, 8·8; $COCH_3$, 18·4 per cent. Calculated for $C_{36}H_{56}O_9$: C, 68·3; H, 8·9; $COCH_3$, 20·4 per cent. Melting point 242°.

The A- and K-aglycones are interrelated. Heating the A-aglycone for 16 hours at 100° with 50 per cent v/v methanol containing 10 per cent w/v sulphuric acid was shown by adsorption chromatography to yield 16 per cent of K-aglycone, and refluxing the A-aglycone with 1 per cent methanolic potassium hydroxide to yield 46 per cent of the K-aglycone. Insufficient I-aglycone was available for analogous tests.

Infra-red Data

The infra-red spectra of aglycones (NaCl prism, KBr phase, concentration 0·3 per cent) suggested that the A- and I-aglycone possess a carbonyl function (C = O stretching absorption at 5·90 μ), which was absent with the K-aglycone. As no carboxyl or ester function could be detected, the carbonyl function is interpreted as a ketone or aldehyde group.

The practically equal intensity of the O–H stretching absorption of I- and K-aglycone was found to be greater than that of A-aglycone. The presence of a cyclic-ether linkage in the K-aglycone is considered probable in connection with the presence of a C–O stretching absorption at 8·85 μ (1130 cm.$^{-1}$) which is lacking in the spectra of the other aglycones and is typical for a C–O–C group[10] in the region between 8·77 and 9·35 μ (1140 and 1070 cm.$^{-1}$). The absence of the intense = C–H bending absorption at 11·30–11·34 μ (882–885 cm.$^{-1}$) which is characteristic of the vinylidene group of the lupeol side chain, indicates that the aglycones are derivatives of the oleanane or ursane series[11–13].

Discussion

Production of isomeric aglycones or artifacts has been observed frequently with steroid saponins[14–16] during the hydrolysis of a single glycoside. But for triterpenoid saponins only aescin[17,18] and the triterpenoid glycoside from *Lemaireocereus stellatus*[19] have been reported as behaving in this manner. The infra-red spectrum of the components isolated from saponin B and the relation between the A- and K-aglycone suggest that they arise from the A-glycone as a result of hydrolysis, and the relation may be expressed thus:

$$\text{A-aglycone} \xrightarrow{+H_2O} \text{I-aglycone} \xrightarrow[\text{tautomerism}]{\text{ring-chain}} \text{K-aglycone.}$$

There were no indications that the K-aglycone is identical with any of the pentacyclic triterpenoids so far isolated.

References

1. Mink, *J. Pharm. Pharmacol.*, 1956, **8**, 1155.
2. Bontems, *Bull. Sci. pharm.*, 1942, **49**, 186.
3. Boiteau, Buzas, Lederer and Polonsky, *Nature, Lond.*, 1949, **163**, 258.
4. Polonsky, Thesis, Paris, 1953.

5. Bhattacharyya and Lythgoe, *Nature, Lond.*, 1949, **163**, 259.
6. Lythgoe and Trippett, *ibid.*, 1949, **163**, 259.
7. Mink, Thesis, Utrecht, 1957.
8. Bhattacharyya, *J. Ind. chem. Soc.*, 1956, **33**, 579; 630.
9. Smit, van Wijk and Ruiter, *Rec. trav. chim.*, 1959, **77**, in the press.
10. Bellamy, *The Infra-red Spectra of Complex Molecules*, Muethen & Co. Ltd., London, 1956, p. 100.
11. Cole and Thornton, *J. chem. Soc.*, 1957, 1332.
12. Arthur, Cole, Thieberg and White, *Chem. Ind.*, 1956, 926.
13. McMurry and Thornton, *Analyt. Chem.*, 1952, **24**, 318.
14. Marker and Rohrmann, *J. Amer. chem. Soc.*, 1940, **62**, 896.
15. Marker, Wagner, Ulshafer, Wittbecker, Goldsmith and Ruof, *ibid.*, 1943, **65**, 1248.
16. Marker and Lopez, *ibid.*, 1947, **69**, 2387.
17. Hofer, Thesis, Zürich, 1950, 64.
18. Ruzicka, Baumgartner and Prelog, *Helv. chim. Acta*, 1949, **32**, 2057; 2069.
19. Djerassi, Liu, Farkas, Lippman, Lemin, Geller, McDonald and Taylor, *J. Amer. chem. Soc.*, 1955, **77**, 1200.