Phytochemistry, 1974, Vol. 13, pp. 1925 to 1927. Pergamon Press. Printed in England.

APTERIN, AN UNUSUAL GLUCOSIDE OF *ZIZIA APTERA**†

WARREN STECK and L. R. WETTER

Prairie Regional Laboratory, National Research Council of Canada, Saskatoon S7N OW9, Canada

(*Received* 23 *January* 1974)

Key Word Index—*Zizia aptera*; Umbelliferae; coumarins; 9-angelyloxycolumbianetin acetate; 9-senecioyloxycolumbianetin acetate; 9-hydroxycolumbianetin glucoside; enzymic hydrolysis.

Abstract—Along with several other coumarin derivatives, a bitter, water-soluble new dihydrofuranocoumarin glucoside was isolated from *Zizia aptera* (Gray) Fern. Its structure was shown to be 8,9-dihydro-8(**S**)-(1-β-D-glucosyloxy-1-methylethyl)-9(**R**)-hydroxy-2H-furo[2,3-h]-1-benzopyran-2-one. It was unattacked by common glucosidases but was cleaved by an enzyme preparation from *Z. aptera* seeds.

Zizia aptera (Gray) Fern. (=*Z. cordata* (Walt.) Koch) is an umbelliferous herb of moist places on the prairies of North America. The plant has a bitter taste which preliminary investigations showed was not associated with alkaloids, but was due to a single water-soluble compound.

The compound (m.p. 236–238°, $[\alpha]_D^{25}$ −229° [0·9, H_2O]) was isolated by column chromatography on mixed charcoal–celite, and recrystallized from water or ethanol. Its solutions were perceptibly bitter at concentrations of 4–5 p.p.m., becoming intensely bitter at 30–50 ppm.

MS indicated the compound had a mol wt of 424·4079 and therefore a molecular formula $C_{20}H_{24}O_{10}$ (calc. 424·4083). The UV absorption spectrum showed a single peak at 325 nm, unshifted by addition of either acid or base. The compound exhibited a very strong blue–violet fluorescence when excited with UV light.

Proton magnetic resonance (PMR) analysis[1,2] in D_2O showed the compound was a *cis*-dihydrofuranocoumarin; the spectrum showed doublets at δ8·30 and 6·66 (*J* 9·5 Hz, H-4 and H-3 resp.), at δ7·97 and 7·35 (*J* 9 Hz, H-5 and H-6 resp.), and at δ5·99 and 4·94 (*J* 6·5 Hz, H-9 and H-8 resp.). In addition 3H singlets were present at δ2·02 and 2·04 (methyl groups), and glycosidic proton signals (6H) were seen at δ3·5–4·1, with an H-1 doublet at 5·34.

Acid hydrolysis (4% H_2SO_4, 1 hr at 95°) gave a single sugar, identified as D-glucose by co-PC (3 systems), PMR and CMR, and oxidation with D-glucose oxidase (E.C.1.1.3.4). The coupling constant of the glucoside PMR doublet at δ5·34, 7·2 Hz, indicated that the glucoside had a β rather than an α configuration.[3] The aglycone recovered, m.p. 179–180°,

* NRCC No. 14031.

† Part III of the series "Prairie Plants". For Part II see Steck, W. (1973) *Phytochemistry* **12**, 2283.

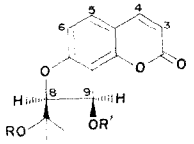
[1] STECK, W. and MAZUREK, M. (1972) *Lloydia* **35**, 418.

[2] NIELSEN, B. E. and LEMMICH, J. (1964) *Acta Chem. Scand.* **18**, 2111.

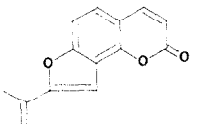
[3] FURBERG, S. and PEDERSEN, B. (1963) *Acta Chem. Scand.* **17**, 1160.

was identified as oroselone (**2**, lit. m.p. 180·5°[4]) by PMR and UV spectra and by co-GC. Oroselone is a characteristic product of acid hydrolysis of diesters of 8-hydroxyisopropyl-9-hydroxydihydrofuranocoumarins.[4,5] The compound from *Zizia* thus appears to be either glucoside **1**a or **1**b.

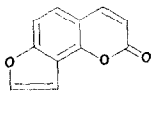
PMR measurements in dimethylsulphoxide-d_6 proved **1**a to be the correct structure. In this solvent proton H-8 gave a doublet at δ4·52 ($J_{8,9}$ 6 Hz), proton H-9 a double doublet at δ5·47 ($J_{8,9}$ 6 Hz, $J_{OH,9}$ 8·5 Hz). The 9-OH proton gave a doublet at δ5·22 ($J_{OH,9}$ 8·5 Hz). These assignments were confirmed by decoupling. The glucose is therefore located at the quaternary carbon of the 8-isopropyl unit, and the glucoside of *Z. aptera* has structure **1**a. The trivial name *apterin* is suggested for this new coumarin. The configuration of the dihydrofuran ring carbon centres is probably 8(**S**), 9(**R**), on the basis of the molecule's optical rotation[2] and from consideration of known dihydrofuranocoumarin configurations.[6] Apterin is the first sugar derivative of hydroxycolumbianetin to be isolated from any natural source.



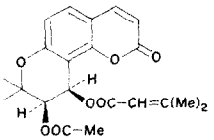
(1a) R = 1 - β - D - glucosyl; R' = H
(1b) R = H; R' = 1 - β - D - glucosyl
(1c) R = R' = H
(1d) R = acetyl; R' = angelyl
(1e) R = acetyl; R' = senecioyl
(1f) R = senecioyl; R' = acetyl



(**2**) Oroselone



(**3**) Angelicin



(**4**) Isosamidin

Apterin exhibits several chemical properties different from those of the familiar hydroxycolumbianetin esters. For example, the glucoside is unattacked by mild bases, including weak alkalies.[4] Acetylation with acetic anhydride and sodium acetate (2hr, 90°) gave a tetraacetate (M^+ = 592) and not a pentaacetate. PMR spectra showed the 9-OH still present (coupling with 9-H, lack of chemical shift of 9-H). This is unusual behaviour for a secondary alcohol and suggests strong steric interaction between the glucose moiety and the C-9 region. Construction of atomic models showed that the β-oxygen bridge is the most likely center for such an interaction; this glycoside link is indeed almost completely enclosed by the gem-methyls and the hydroxyl group at C-9. Of related interest is the resistance of the glucoside to enzymic cleavage: it proved resistant to enzymic hydrolysis by emulsin (β-glucosidase) or maltase (α-glucosidase). Preliminary experiments on cell-free extracts of various plants and tissues also proved to be negative. However, cell-free extracts of *Z. aptera* seeds contained a glycosidase that hydrolyzed apterin. On the basis of this discovery, a larger scale enzymic hydrolysis of 0·1 g apterin was carried out. The hydrolysis products proved to be glucose and vaginol (**1**c) (MS, UV, PMR); this last compound has been reported[7] from *Selinum vaginatum* C. B. Clarke. Physical data on the apterin aglycone were concordant with the data on vaginol, confirming the structure **1**a for apterin. It is notable that whereas vaginol is a very minor component of *S. vaginatum*, the glucoside apterin is found abundantly in *Z. aptera*, and may offer a good starting point for synthesis in the vaginol series.

[4] MITSUHASHI, H. and ITOH, T. (1961) *Chem. Pharm. Bull. (Tokyo)* **9**, 170.
[5] HALPERN, O., WASER, P. and SCHMID, H. (1957) *Helv. Chim. Acta* **40**, 758.
[6] LEMMICH, E., LEMMICH, J. and NIELSEN, B. E. (1970) *Acta Chem. Scand.* **24**, 2893.
[7] RAJENDRAN, K., MESTA, C. K., PAKNIKAR, S. K. and BHATTACHARYYA, S. C. (1970) *Indian J. Chem.* **8**, 200.

Besides apterin, *Z. aptera* extracts yielded a number of other heterocyclic compounds. Large quantities of rutin were obtained and six ether-soluble coumarins structurally related to apterin were isolated and characterized. These were edultin (**1d**), isosamidin (**4**) and 9-(**R**)-senecioyloxycolumbianetin acetate (**1e**). An inseparable mixture of 9-(3-methylbutyryloxy)- and 9-(2-methylbutyryloxy)-columbianetin acetate was characterized by PMR. The angular furanocoumarin angelicin (**3**), obtained in small quantities from diverse fractions, was almost certainly an artefact.

EXPERIMENTAL

Isolation of apterin. Plant material was collected in July 1972 and again in July 1973, 10 km North-East of Saskatoon. The air-dried aerial parts (600 g) were powdered and added to boiling methanol (4 l). After subsequent filtration the filtrate was adjusted to 80% methanol and partitioned twice with hexane. Then the alcoholic soln was mostly evaporated, diluted with H_2O and extracted with methylene chloride. The residual H_2O soln deposited, on standing 24 hr, 8·6 g pure rutin, which was filtered off and identified by comparisons with authentic material. The filtrate from this last filtration, intensely blue-fluorescent, was conc, then chromatographed on a 2·5 × 30 cm column of 1:1 celite: Darco G charcoal. Elution with aq alcohols gave syrupy material, then with 95% ethanol and with ethanol: C_6H_6 gave 2·1 g crude apterin. After 3 recrystallizations from H_2O, 1·6 g glucoside, m.p. 236–238°, was obtained. Apterin was bitter to all tasters at the 4 μg level.

Isolation of other coumarins. The methylene chloride extract was evaporated and the residue, 6 g, was chromatographed on a 400 g column of silicic acid (Mallinckrodt), using ether–hexane (1:1) for development. The first compounds to emerge were 9-(2-methylbutyryloxy)- and 9-(3-methylbutyryloxy)-columbianetin acetate, unseparated but readily characterized by differential PMR spectra. Next obtained was 0·2 g edultin (**1d**), m.p. 152–156° (lit. m.p. 144–146°[4]), UV and PMR spectra[1] identical with those of authentic material.[8] Third to elute was 0·4 g 9-(**R**)-senecioyloxycolumbianetin acetate (**1e**), m.p. 133–3·5° from 70% methanol. λ_{max} (EtOH) 325 nm; M^+ 386, with appropriate fragmentation; $[\alpha]_D^{25}$ −54·8° (0·685, $HCCl_3$). Direct PMR comparison with 9-(**R**)-acetoxycolumbianetin senecioate (**1f**), m.p. 125·5–6·0°, showed small but definitive differences, thus locating the ester moieties.[1] A fourth coumarin gave a PMR spectrum completely identical with that of *isosamidin* (3'-acetyl-4'-senecioyl-*cis*-khellactone, **4**).[9] *Angelicin* was isolated in traces from several different fractions, and was the only major peak recorded on attempted GC (SE-30, 200°) of the various dihydrofuran derivatives. It appears to be an artefact resulting from thermal decomposition of vaginol esters. Later fractions eluted with ether–ethyl acetate contained mixtures of unidentified vaginol monoesters (believed to be 9-dihydroangelyl and 9-dihydrosenecioyl on the basis of PMR analysis).

Enzymic hydrolysis of apterin. Freshly-gathered *Z. aptera* seed (5 g) was extracted into 75 ml of extraction soln in an Omni-mixer. The extraction solution was 200 ml of 0·1 M phosphate buffer, pH 7·0, containing 31 mg of dithiothreitol and 2·34 g of NaCl. To the 75 ml of solution 10 g of Polyclar AT (polyvinylpyrrolidone) was added prior to grinding. The supernatant liquid was fractionated with solid ammonium sulfate and the protein precipitate obtained between 40 and 80% saturation contained the glycosidase activity. This protein fraction hydrolyzed not only apterin but also umbelliferyl glucopyranoside and its 4-methyl analog. The large scale experiment was set up by dissolving 100 mg of apterin in 25 ml of 0·01 M phosphate buffer, pH 7, and adding fractionated enzyme equivalent to about 2·5 g of *Zizia* seed. After 5 days' hydrolysis the aglycone was extracted from the mixture with CH_2Cl_2. Evaporation of the solvent left colourless crystals of vaginol, m.p. 163–165° from alcohol (lit. 164–165°[7]). M^+ 262. λ_{max} (EtOH) 325 nm. PMR spectrum ($CDCl_3$): doublets, J 9·5 Hz, at δ6·15 [H-3] and 7·56 [H-4]; doublets, J 8·5 Hz, at δ6·76 [H-6] and 7·29 [H-5]; doublet $J_{8,9}$ 6·5 Hz, at δ4·34 [H-8] coupled to a multiplet at 5·80 [H-9]; singlets at δ1·50 and 1·56 [gem-methyls] and at 3·97 [OH]. A poorly resolved OH doublet at δ4·78 was reduced to a singlet by decoupling at the H-9 peak [3079 Hz].

Acknowledgements—We thank Messrs. B. K. Bailey and J. Dyck for expert technical assistance; Professor M. Nakazaki (Osaka University) for an authentic sample of edultin; and Dr. B. E. Nielsen (Royal Danish School of Pharmacy) for 9-(**R**)-acetoxycolumbianetin senecioate. *Z. aptera* was identified by Dr. V. L. Harms, University of Saskatchewan Herbarium, Saskatoon, Canada.

[8] Nakazaki, M., Hirose, Y. and Ikematsu, K. (1966) *Tetrahedron Letters* 4735.
[9] Lemmich, J., Lemmich, E. and Nielsen, B. E. (1966) *Acta Chem. Scand.* **20**, 2497.