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1,4-BENZOXAZINE DERIVATIVES IN PLANTS
A NEW TYPE OF GLUCOSIDE FROM ZEA MAYS

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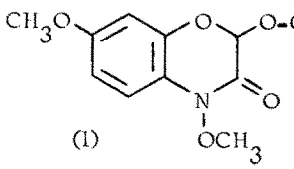
Through the study of 1,4-benzoxazine in plants a new glucosidic derivative was identified. It appears in corn, wheat and Coix Lachryma Jobi, mostly in roots, where it is the main component of the benzoxazine glucoside type. It was isolated from corn by gel filtration on Sephadex G-10 and in a previous publication it was denoted as "III-Glc."(1).

For this substance the structure 2-(2-hydroxy-4,7-dimethoxy-1,4/2H/-benzoxazin-3-one)-ß-D-glucopyranoside (I) is now suggested. Through crystallization from ethanol diluted with benzene to 5% of ethanol and recrystallization from absolute ethanol colourless needles of intensively bitter taste of melting point 142-4°C, UV $\lambda_{max}(H_2O)$ 264 nm, shoulder 282 nm, $\varepsilon^{264}_{H_2O}$ = 9 500 were obtained; they did not form a colour complex with $FeCl_3$. In descending paper chromatography in system n-BuOH, EtOH, H_2O (5:1:4), Whatm. No 3, the value R_f 0.5 was observed. Anal. calc. for $C_{16}H_{21}NO_{10}$: C,49.61;H,5.46;N,3.61; found: C,50.00;H,5.42;N,3.72; the methoxy content calc. 16.02%, found 15.82%. On infrared spectra the ketone stretching vibration at 1705 cm^{-1} appearing in 1,4-benzoxazin-3-one derivatives could be observed. The NMR spectrum was obtained by using deuterated dimethysulphoxide as the solvent and hexamethyldisiloxane as an internal standard. The proton C-5 absorbs at 7.05 ppm (doublet, J=8.5 cps), C-8 at 6.73 ppm (doublet, J=3cps), C-6 at 6.60 ppm (quartet), C-2 at 5.80 ppm, anomeric proton C-1 at 4.61 ppm (doublet, J=7 cps) and absorption due to the protons of two methoxy groups are at 3.87 and 3.72ppm (aromatic). The signal of amide proton was not found. NMR spectrum of I is similar to that of hydroxam form 2-(2,4-dihydroxy-7-methoxy-1,4/2H/-benzoxazin-3/4H/-one)-ß-D-



(I)

glucopyranoside (DIMBOA-Glc.) (2). Only the signal of proton C-5 in which there occurs a shift to the down field due to the influence of protons of the methoxy group upon nitrogen is different. An opposite effect was observed in "1-Glc".(1,3), in which the protons of benzene ring (C-5, C-6, C-8) appear at a higher field than in 2-(2-hydroxy-7-methoxy-1,4/2H/-benzoxazin-3/4H/-one)-ß-D-glucopyranoside (HMBOA-Glc.)(2).

In order to gain more evidence for the structure I , the compound (5mg) was reduced in aqueous solution by sodium hydrosulphite (50°C, six hours; cf.(2)). After the paper chromatography of the reaction mixture the product R_f 0.4 (yield cca 80%, by photometry) was eluated with water on chromatographic elution device (4) and refined further by gel filtration on Sephadex G-10. R_f, elution volume and UV and NMR spectra are the same as in HMBOA-Glc. Mass spectrum is also very similar to that of HMBOA-Glc. In further experiment, DIMBOA-Glc.(40 mg, in ether solution) was methylated by diazomethane (4 days at 20°C); the product, purified by paper chromatography, does not react with $FeCl_3$ and has the same R_f, UV and NMR spectra as I .

The structure of I explains the relatively high lipophilic character as compared with the other derivatives isolated so far, and, further, the negative reaction with $FeCl_3$. The low m.p. corresponds to N-substituted compound that generally have lower m.p. than the substances with amidic group (5). Substance I is not stable. At room temperature in moist air it gets brown and substances of R_f 0.4 and 0.3 occur. Its instability also appears in enzymic splitting with ß-glucosidase of corn. At room temperature several components absorbing in UV region appear out of which the strongest is the component of R_f 0.9, $\lambda_{max}(H_2O)$ 229 and 284 nm. Through enzymic splitting at 0°C this substance is formed almost without any by-products. Since this product has a similar absorption in the UV region as 6-methoxy-2(3)-benzoxazolinone, it can interfere in photometric determination of the DIMBOA-Glc. content in plants (6). When the herbicide atrazine is applied upon corn seedlings, the decrease in concentration of I can be observed.

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