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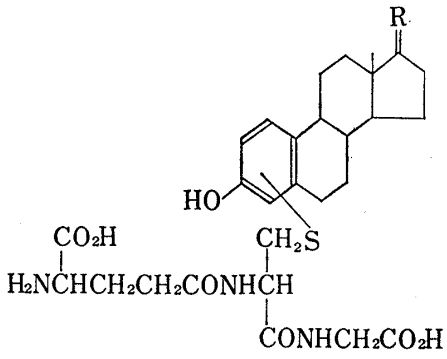
Estrogen-Glutathione Conjugate : Metabolite of 3-Deoxyestrone in Rat

Recently considerable attentions have been drawn to the problems associated with the physiological significance of the steroid conjugate. We now wish to report the separation and characterization of estrogen-glutathione conjugate excreted in rat urine after large dosage of 3-deoxyestrone.

A suspension of 3-deoxyestrone (100 mg) in Tween 80 was orally given to each of ten adult male rats (Wistar strain), and at the same time four of them were injected intraperitoneally with 18.7 μCi of 3-deoxyestrone-6,7-^{3}H(68.4 μCi/μmole)[1] dissolved in 1 ml of 10% ethanolic saline. The urine was collected for the following 5 days during which the radioactive metabolites corresponding to 14% of the dose were excreted.

The urine specimen was extracted with ether and submitted to hydrolysis with beef-liver β-glucuronidase and then solvolysis in the usual manner.[2] The radioactivity amounted to 18% of the total urinary metabolites still remained in the unhydrolyzable fraction. The water-soluble material was adsorbed on Amberlite XAD-2 resin and washed with water for removal of the non-steroidal substance. The crude metabolite eluted with methanol was then chromatographed on Sephadex G-25 and the radioactive fraction was collected. The labeled metabolite behaved like a peptide-conjugate on thin-layer plate (*Rf* 0.54 BuOH–AcOH–H_2O (4:1:1), silica gel G) exhibiting reddish purple coloration with conc. sulfuric acid and purple with ninhydrin. In order to facilitate further purification this water-soluble compound was transformed into the dinitrophenyl (DNP) derivative by the reaction with 2,4-dinitrofluorobenzene in the usual way. The radioactive product became soluble in organic solvent such as ethyl acetate and ran as a single yellow spot on thin-layer chromatogram (*Rf* 0.67 *n*-PrOH–28% NH_4OH (9:1), silica gel G). Upon hydrolysis with 6N hydrochloric acid the pruified DNP-derivative yielded glycine and DNP-glutamic acid, which were readily characterized by thin-layer chromatography. These results lent a support to assign the glutathione conjugate to the water-soluble metabolite. Elucidation of the steroidal moiety in this peptide conjugate was achieved by treatment with Raney nickel in 0.5 N acetic acid. Thus it proved that the desulfurization product consisted of estradiol and estrone in ratio of *ca.* 3 to 1 according to the thin-layer and gas-liquid chromatography (3% SE-30) after trimethylsilylation. Characterization of the free steroids was further confirmed by the isotope dilution method. These findings together led to the conclusion that the estrogens should be linked with tripeptide by a carbon-sulfur bond as shown in I.



Ia : R = ⟨OH, H⟩

Ib : R = O

Kuss[3] and Jellinck, *et al.*[4] observed independently that estradiol was converted by rat liver into a water-soluble metabolite whose structure was assumed to be a glutathione conjugate

1) Prepared in this laboratory from estrone-6,7-^{3}H (The Radiochemical Centre, England) through 1-phenyl-5-tetrazolyl ether.
2) S. Burstein and S. Lieberman, *J. Biol. Chem.*, **233**, 331 (1958).
3) E. Kuss, *Z. Physiol. Chem.*, **348**, 1707 (1967); **349**, 1234 (1968); **350**, 95 (1969).
4) P. H. Jellinck, J. Lewis, and F. Boston, *Steroids*, **10**, 329 (1967).

with catechol-type or quinone-type estrogen. It should be now emphasized that the steroidal moiety in the new peptide conjugate is not 2-oxygenated estrogen but estradiol or estrone itself. The proposed mechanism by these investigators involving the additive condensation of the thiol with *o*-quinone is not applicable and hence an alternative explanation must be required for the formation of this novel metabolite. In our previous study the occurrence of "NIH shift" during aromatic ring hydroxylation was observed with 3-deoxyestrone.[5] If arene-oxide would be a metabolic intermediate common to both hydroxylation and glutathione conjugation,[6] the introduced position of the thiol should be C-2 or C-4 in the steroid nucleus.

Further studies in progress in this laboratory may provide the data necessary for the more rigorous characterization as well as the formation mechanism of the estrogen-glutathione conjugate.

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5) T. Nambara, M. Numazawa, and S. Akiyama, *Chem. Pharm. Bull.* (Tokyo), **19**, 153 (1971).
6) D. M. Jerina, J. W. Daly, and B. Witkop, *J. Am. Chem. Soc.*, **90**, 6523 (1968); D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman-Nierenberg, and S. Udenfriend, *ibid.*, **90**, 6525 (1968).

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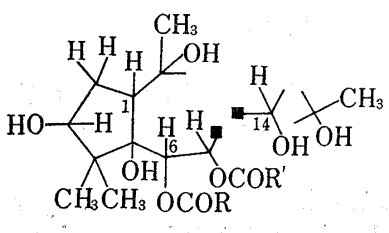
Stereostructure of Asebotoxin IV and V, Toxins of *Pieris japonica*

We have previously reported the isolation and structural elucidation of three new toxic diterpenoids, asebotoxin I, II, and III, from the flowers of *Pieris japonica* D. Don (Ericaceae), a poisonous tree in Japan.[1,2] Continuation of our work has resulted in the further isolation of two novel diterpenoids for which the names asebotoxin IV and V (A-IV and A-V) are proposed.

A-V, $C_{25}H_{40}O_9$, mp 280—281°, was shown by spectral properties to have two tertiary methyls (0.99, 1.48 ppm), two tertiary methyls on hydroxyl-carrying carbons (1.48, 1.82 ppm), hydroxyls (3490, 3280 cm^{-1}) two of which are secondary (3.85, 5.21 ppm), a secondary O-acetyl (1714 or 1729, 1253 cm^{-1}, 2.05, 6.14 ppm) and a secondary O-propionyl (1729 or 1714cm^{-1}, 1.12, 2.45, 6.09 ppm). Further analysis of the nuclear magnetic resonance (NMR) spectrum revealed that A-V possesses the partial structure A in which the C-1 and C-6 hydrogens and the C-1 and C-14 hydrogens are sit-



A: R=CH_3, R′=CH_2CH_3 or *vice versa*
▪ : denotes a quaternary carbon

1) H. Hikino, K. Ito, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **17**, 854 (1969).
2) H. Hikino, K. Ito, T. Ohta, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **17**, 1078 (1969).