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ALKALOIDS OF Aconitum coreanum

V. 13-ACETYL-14-HYDROXY-2-ISOBUTYRYLHETISINE

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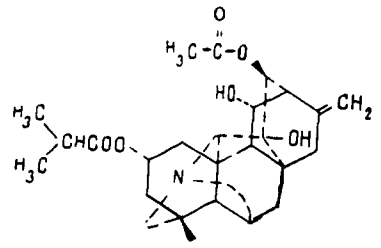
The isolation from the epigeal part of Aconitum coreanum (Levl.) Rapaics of a number of alkaloids of the hetisine and atisine types has been reported previously [1, 2].

Continuing the chromatographic separation of the hexane–ether fractions, we have isolated a base with the composition $C_{26}H_{35}NO_6$, mp 181-182°C (from acetone), $[\alpha]_D$ +58° ($CHCl_3$) (I). The IR spectrum of (I) had absorption bands of hydroxy (3555-3430 cm^{-1}), ester (1745, 1735 cm^{-1}), and exomethylene (1680 cm^{-1}) groups. The following were the main peaks in the mass spectrum of (I), m/z (%): 457 (M^+, 52); 415 [$(M - 42)^+$, 28], 414 [$(M - 43)^+$, 100], 398 [$(M - 59)^+$, 49], 370 [$(M - 87)^+$, 2]. These facts, and also the presence in the PMR spectrum of the signals of the protons of exomethylene, 18-methyl, isobutyryl, and acetyl groups and its close similarity to the PMR spectrum of 14-hydroxy-2-isobutyrylhetisine (Guan-Fu base Z) [3] permitted the assumption that the compound isolated was an acyl derivative of 14-hydroxy-2-isobutyrylhetisine.

A comparative analysis of the PMR spectra of (I) and (II) [3, 4] enabled an assignment to be made of the signals in the PMR spectrum of (I) (Tesla 567 A, 100 MHz, $CDCl_3$, O–HMDS, δ, ppm): 5.12 (br.s, H-2); 4.99 (br.s, H-13); 4.89 and 4.68 (br.s, 1H each, 2H-17); 4.19 (d, J = 9 Hz, H-11); 3.32 (s, H-20), 3.07 (br.s, H-6), 2.88 (d, J = 16 Hz, H-1α); 2.83 and 2.47 (d, 1H each, J = 12 Hz, H-19β and H-19α); 2.55 (d, J = 4 Hz, H-12); 2.40 (m, H-2'); 2.06-1.64 (m, 2H-15, H-9, H-1β, H-7α, H-3α); 1.97 (s, CH_3CO), 1.56 (d.d, J = 15.5; 4 Hz, H-3β); 1.49 (s, H-5), 1.33 (d.d, J = 14, 2 Hz, H-7β); 1.13 and 1.09 (d, 3H each, J = 6.5 Hz, $-C(CH_3)_2$); 0.96 (s, 3H, CH_3) – and on this basis to conclude that (I) had the structure of 13-acetyl-14-hydroxy-2-isobutyrylhetisine.



A direct chromatographic comparison with an authentic sample of 13-acetyl-14-hydroxy-2-isobutyrylhetisine (Guan-Fu base F) isolated from the plant Aconitum coreanum growing in China showed their identity. We thank Jinghan Liu for the information sent [5] and for a sample of Guan-Fu base F.

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ISOLATION OF HIGHLY SPECIFIC PROTEINS FROM COTTON SEEDS OF VARIETY C-6030

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In a comparative investigation of the seeds of cotton plants of the varieties Tashkent-1 (G. hirsutum) and C-6030 (G. barbadense) by electrophoresis in PAG [1], individual proteins specific for this species were detected with R_f 0.37 and 0.48 for G. barbadense (B. 0.37 and B. 0.48) and with R_f 0.43 and 0.51 for G. hirsutum (H. 0.43 and H. 0.51) [2]. In order to elucidate the differences in their physicochemical characteristics that are responsible for their high specificity it was necessary to isolate and purify them.

The individual proteins from cotton seeds of the variety C-6030 were isolated from the acetone-defatted flour. After three extractions of the flour with distilled water, which removed the bulk of the water-soluble proteins, the flour was stirred for an hour with 0.05 M Tris-HCl buffer, pH 8.9, in a ratio of 1:10 (weight/volume). The resulting suspension was centrifuged at 3000 rpm (K-70) for 30 min, and the supernatant was treated with dry ammonium sulfate to 80% saturation and was then kept in the refrigerator until a precipitate had formed. The precipitate contained the accompanying high-molecular-mass proteins. It was separated off by centrifugation, and the supernatant was again treated with dry ammonium sulfate, to 100% saturation, and was left in the refrigerator. The protein precipitate that deposited, enriched with fractions of the highly specific proteins B. 0.37 and B. 0.48, was separated off by centrifugation and was dissolved in 0.05 M Tris-HCl buffer, pH 8.9, and the solution was dialyzed against water and was lyophilized. Electrophoresis in PAG showed that the fraction obtained at 100% saturation contained other proteins besides the highly specific proteins.

The further purification of proteins B. 0.37 and B. 0.48 was performed by gel filtration on a column of Sephadex G-100. On chromatography in 0.05 M NH_4HCO_3 the proteins were separated into three peaks. After lyophilization and electrophoresis of the fractions obtained it was found that the highly specific proteins of G. barbadense had issued from the column in the second peak, without being separated from one another.

The highly specific proteins B. 0.37 and B. 0.48 were separated by preparative isoelectric focusing. The isoelectric points of these proteins were first determined by analytical isofocusing, which was conducted on a Multiphor instrument (LKB) in the prepared PAG gels associated with it. Pieces of filter paper were impregnated with 10 μl of protein solution (concentration 2 mg/ml) and were deposited on a gel with a pH gradient of from 3.5 to 9.5. A standard set of proteins with known isoelectric points (pI – Marker Proteins; Protein Test Mixture 9, Serva) was deposited in parallel. After cooling to 8-10°C, isofocusing was carried out at a voltage of 200-500 V for 2 h. The gels were fixed and stained and were then washed free from the excess of dye. The results of isofocusing showed that protein B. 0.37 had an isopoint of 6.2, and protein B. 0.48 one of 5.8.

Preparative isoelectric focusing was conducted in a LKB vertical column in a 5-60% gradient of sucrose solution containing ampholines with pH 5-7 in a concentration of 1%. The

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