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25 March 1969

Institute of the Chemistry of Plant Substances AS UzSSR

UDC 547.944/945

ALKALOIDS OF PEDICULARIS

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Khimiya Prirodnykh Soedinenii, Vol. 5, No. 4, pp. 334-335, 1969

From the epigeal part of P. rhinanthoides Schrenk., collected on July 21, 1966, in the flowering period in the gorge of the R. Nura, KirgSSR, by chloroform extraction we have obtained 0.3% of total alkaloids.

The ethereal fraction of the total alkaloids was treated with acetone, giving 0.04% of plantagonine [1]. By separating the mother liquor on a column of alumina [eluant: ether–chloroform (9 : 1)] we obtained a liquid base with R_f 0.67 [in the butan-1-ol–water–acetic acid (20 : 20 : 1) system], $[\alpha]_D^{20}$ +5.9 (c 0.508; ethanol), $C_{10}H_{13}NO$, mol. wt. 163 (mass spectroscopy). The picrate has mp 151-152° C (water). IR spectrum: λ_{max} 263, 270 mμ (log ε 2.74, 2.70).

The IR spectrum of the base has absorption bands at 3400-3200 cm^{-1} (OH), 2960 cm^{-1} (C–CH_3), 1595 cm^{-1} (pyridine ring), and 895, 850, and 815 cm^{-1}. Oxidation of the base with potassium permanganate in an alkaline medium added two oxygen atoms with the formation of an acid with mp 218-220° C (decomp.). A mixture of this acid with plantagonine showed no depression of the melting point. Thus, the base that we have isolated is the dextrorotatory form of the known alkaloid *l*-tecostidine [2].

From the epigeal part of P. olgae Rgl., collected on June 15, 1968, in the flowering period in the village of Saed, TadzhSSR, we have obtained 0.65% of total alkaloids from which we have isolated 0.11% of plantagonine.

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25 March 1969

Institute of the Chemistry of Plant Substances, AS UzSSR

UDC 547.962

MALATE DEHYDROGENASE FROM COTTON SEED

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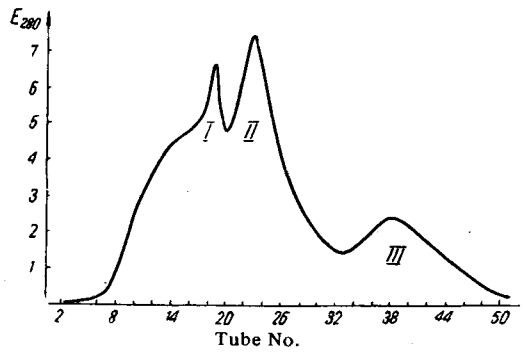
Khimiya Prirodnykh Soedinenii, Vol. 5, No. 4, pp. 335-336, 1969

From the seeds of the cotton plant of variety 108-F, we have isolated a fraction possessing malate dehydrogenase activity.

The seeds, freed from their coating and ground, were defatted [1]. The resulting acetonic powder (100 g) was mixed with 0.01 M phosphate buffer, pH 7.4 (1 : 10) containing 0.005 M EDTA and 0.005 β-mercaptoethanol. The extracts were centrifuged at 6000 rpm for 30 min. The supernatant liquid was fractionally precipitated with ammonium sulfate. The precipitate obtained at 20% saturation was filtered off with suction and the supernatant liquid was brought to 50% saturation. Then the precipitate was dissolved in the minimum amount of 0.1 M phosphate buffer, pH 7.4, containing EDTA and β-mercaptoethanol and was passed through a 2.5 × 45 cm column containing Sephadex G-25 equilibrated with the same buffer. The fractions containing protein were combined and the percentage protein content was



Separation of the protein fraction on a column of Sephadex G-100.

determined by the Warburg-Christian method [2] and investigated for malate dehydrogenase, lactate dehydrogenase, and glutamate dehydrogenase activity by the spectrophotometric method [3].

The separation of the resulting protein fraction was carried out on a column of Sephadex G-100 (5 × 100 cm, rate of elution 24 ml/hr) equilibrated with 0.1 M phosphate buffer, pH 7.4. Elution was carried out with the same buffer and the fractions corresponding to the individual peaks were combined (figure). A high specific malate dehydrogenase (MDH) activity was found in the second peak (figure, peak II). These fractions also had lactate dehydrogenase (LDH) activity, while in the third peak (peak III) only LDH was found. The fractions corresponding to the second peak were dialyzed for a day against 0.01 M phosphate buffer, pH 7.6, and were transferred to a column of DEAE-cellulose (2.5× 25 cm) equilibrated with the same buffer. Separation was carried out by means of a stepped gradient of the same phosphate buffer with concentractions of NaCl of from 0 to 0.3 M. According to the graph of the elution of protein, we obtained one low-intensity peak, eluted at low concentrations of salt, and an intense peak eluted at high concentrations.

The fractions corresponding to the second peak and containing MDH were combined, desalted, and freeze-dried.

The protein fractions contained in these stages of isolation and purification were investigated by electrophoresis in acrylamide gel [4]. In the initial extract, five electrophoretic components were found. In the first peak after separation on the Sephadex G-100 column there was one component, in the second peak one component, and in the third peak three components; after separation on the DEAE cellulose column there was a single peak.

All the experiments were carried out at +4° C.

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7 July 1968

Institute of the Chemistry of Plant Substances AS UzSSR