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L-(+)-ISOLEUCINE BETAINE IN *CANNABIS* SEEDS*

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Key Word Index—*Cannabis sativa*; Moraceae; Indian hemp; isolation of quaternary bases; isoleucine betaine; synthesis.

Abstract—A new base, L-(+)-isoleucine betaine, has been isolated from *Cannabis* seeds. The structure was determined by spectroscopic methods and confirmed by synthesis.

INTRODUCTION

IT IS about 100 years since Preobraschensky[1] published on the possible presence of a nitrogenous base in *Cannabis*. Some years later, Jahns[2] identified choline from Indian hemp and Schulze and Frankfurt[3] reported the presence of trigonelline in hemp seeds. In 1960 Obata *et al.*[4] identified piperidine and Klein *et al.*[5] described the isolation of four alkaloids, called cannabamines A, B, C and D. Samrah *et al.*[6,7] and Aguar[8] provided chromatographic evidence for the presence of alkaloidal substances in hemp.

Our earlier investigations[9,10] confirmed the findings of Jahns and Schulze and Frankfurt, while trigonelline was also found in the stems of hemp plants. Although we could not confirm the results of Obata, evidence was obtained for the presence of several unidentified quaternary nitrogen bases. The present paper concerns the isolation and identification of one of these compounds from hemp seeds.

RESULTS AND DISCUSSION

The purified base, which could not be obtained in a crystalline form, showed a positive reaction towards Dragendorff's reagent. The component was optically active, $[\alpha]_D^{20}$ +18·0° (*c* 1, $CHCl_3$). The IR spectrum showed main absorptions at 3340, 2960, 1625, 1490, 1450, 1390, 1030, 970, 960, 920 and 860 cm^{-1}, while acidification of the sample caused a remarkable shift of the 1625 cm^{-1} band to 1710 cm^{-1}, simultaneously with the appearance of the typical broad absorption of carboxylic acids at the appropriate high wave-number, and

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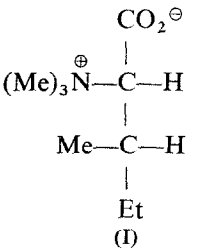
with the appearance of a strong absorption at 965 cm^{-1} which could be ascribed to an antisymmetrical stretching of a trimethylammonium group.[11]

The 100 MHz proton magnetic resonance spectrum (in D_2O) showed resonances at δ1·02 (*t*, *J* 7·3 Hz, 3H), 1·06 (*d*, *J* 7·0 Hz, 3H), 1·58 (*m*, *J* 7·0 Hz, 2H), 2·11 (*m*, 1H), 3·24 (*s*, 9H) and 3·67 (*d*, *J* 1·1 Hz, 1H) ppm. Double resonance experiments indicated the presence of a secondary butyl group. The resonance at δ3·24 ppm was ascribed to the trimethylammonium group which could be confirmed by Hofmann degradation, yielding trimethylammonium hydrochloride which was identified in comparison with an authentic sample.



(I)

Mass spectrometric and elemental analysis of this type of compound is notoriously difficult because of the ease of fragmentation under high vacuum and their very strong hygroscopic character. However, PMR and IR determined functional groups suggested the structure of isoleucine betaine (I) as being in accordance with the analytical data. This compound was therefore synthesized by reaction of trimethylamine with 2-bromo-3-methylpentanoic acid.[12] The spectroscopic data of racemic isoleucine betaine were in good agreement with those of the natural product. Therefore, we decided to synthesize L-isoleucine betaine, the most probable natural optical isomer, by a modification of the method of Abderhalden.[13] The synthesized L-isoleucine betaine showed optical activity, $[\alpha]_D^{20}$ +17·5° (*c* 1, $CHCl_3$) and possessed PMR and IR spectra which were identical with those of the natural product.

During the above reaction the formation of the dextro rotatory isomer, D-alloisoleucine betaine, due to the Walden inversion[13,14] was possible, and we therefore synthesized L-(+)-isoleucine betaine by an alternative route. Methylation of L-(+)-isoleucine first by formaldehyde and platinum oxide and hydrogen[15] and next with methyl iodide in weak basic medium produced a product which showed complete identity with the isolated compound and the one obtained by the previous synthesis. One may thus conclude that in the former case no Walden inversion occurred.

Although earlier produced synthetically,[16] this is apparently the first report of the natural occurrence of L-(+)-isoleucine betaine.

Preliminary pharmacological assays on analgesic, hypothermal, rotating rod and toxicity effects of isoleucine betaine on mice did not reveal any acute symptoms (LD_{50} 2 g/kg body weight of mice).

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EXPERIMENTAL

The IR spectra were recorded on a Beckman IR 8 instrument; the PMR spectra were recorded on Varian A60 and HA100 spectrometers; the optical rotation was measured on a Perkin Elmer 141 Polarimeter.

Extraction and purification. 300 kg of ground fresh hemp seed, variety Fibrimon, were extracted (portion-wise) with 2200 l. EtOH. The combined extracts were acidified with 200 ml glacial HOAc in 50 l. H_2O. The extract was then concentrated to about 20 l. under vac. (temp. $<40°$). The aq. soln was exhaustively extracted with C_6H_6 and the C_6H_6 was removed from the filtered aq. layer. An excess of Kraut reagent[9] was added to the soln, the reddish ppt (560 g) was filtered and suspended portion-wise (100 g) in H_2O (750 ml). The suspension was decomposed by H_2S. After filtration, the filtrates were concentrated to 0·25 of the vol. and subsequently shaken with freshly prepared AgCl. Filtration provided solns which were concentrated under vac.

The residue was further purified by column chromatography on cellulose (three consecutive columns, 2·1 kg of cellulose powder Macherey and Nagel MN 100 per column, 480 g per l. of column vol.), eluted with *n*-BuOH, saturated with H_2O. The fraction with an R_f of *ca.* 0·45 (TLC, cellulose MN 300 eluent *n*-BuOH, saturated with H_2O; Dragendorff's reagent for detection) was chromatographed on a column (30 × 3 cm) of basic alumina using MeOH as eluent. The compound with R_f 0·30 (TLC, basic alumina/MeOH) was purified on a $CaHPO_4.2H_2O$ column, which was first washed with *n*-pentane, followed by $CHCl_3$, giving 103 mg of isoleucine betaine.

Synthesis of isoleucine betaine. In a sealed tube 4 g 2-bromo-3-methylpentanoic acid (prepared according to the procedure of Ehrlich[12]) and 50 ml 30% aq. trimethylamine were kept for 5 days at 35°. After addition of 1·6 g NaOH the mixture was concentrated under vac. The residue was acidified to pH 1 with 6 N HBr, concentrated to a viscous syrup and finally extracted with *n*-BuOH. The extract was concentrated, giving 4 g (84·4%) isoleucine betaine HBr, m.p. 204–205° (recrystallized from *n*-BuOH). IR (KBr): 1730, 1495, 1450, 1420, 1355, 1245, 1190, 1160, 1130, 970, 865, 850 and 725 cm^{-1}. 60 MHz PMR (in D_2O): δ 0·98 (*t*, *J* 7·5 Hz, 3H), 1·04 (*d*, *J* 7·0 Hz, 3H), 1·59 (*m*, *J* 7·0 Hz, 2H), 2·26 (*m*, 1H), 3·27 (*s*, 9H), 4·04 (broad *s*, 1H) and 4·66 (HDO) ppm. A soln of 1 g isoleucine betaine HBr in 5 ml H_2O was shaken for 1 hr with Ag_2O. After filtration, the soln was concentrated under vac. The remaining oil crystallized after prolonged drying (P_2O_5) at 10 mm. IR (KBr): 3330, 2975, 1625, 1490, 1460, 1450, 1390, 1050, 965, 920 and 860 cm^{-1}. 60 MHz PMR (in D_2O). δ 1·00 (*t*, *J* 7·0 Hz, 3H), 1·04 (*d*, *J* 6·5 Hz, 3H), 1·56 (*m*, 2H), 2·13 (*m*, 1H), 3·22 (*s*, 9H), 3·61 (*d*, *J* 1·5 Hz) and 3·66 (*d*, *J* 1·0 Hz)(1H) and 4·67 (HDO) ppm.

Synthesis of L-(+)-isoleucine betaine. (A) L-(+)-Isoleucine was converted into the corresponding 2-bromo-3-methylpentanoic acid by the method of Abderhalden.[13] The bromoproduct was worked up in the same way as the racemic compound. L-(+)-isoleucine betaine HBr, m.p. 206·5–208°. IR: the same as for the racemic compound. 60 MHz PMR (in D_2O): δ 0·95 (*t*, *J* 7·0 Hz, 3H), 1·01 (*d J*, 6·5 Hz, 3H), 1·54 (*m*, 2H), 2·24 (*m*, 1H), 3·22 (*s*, 9H), 4·01 (*d*, *J* 1·0 Hz, 1H) and 4·66 (HDO) ppm. L-(+)-Isoleucine betaine. IR. The same as for the racemic compound. 60 MHz PMR (in D_2O): δ 0·99 (*t*, *J* 7·0 Hz, 3H), 1·05 (*d*, *J* 6·5 Hz, 3H), 1·57 (*m*, 2H), 2·10 (*m*, 1H), 3·23 (*s*, 9H), 3·65 (*d*, *J* 1·0 Hz, 1H) and 4·67 (HDO) ppm. (B) *N,N*-Dimethyl-L-isoleucine was prepared according to the synthesis of Suyama[15] by shaking an HOAc soln of L-(+)-isoleucine with formaldehyde and platinum oxide in an atmosphere of H_2. *N,N*-Dimethyl-L-isoleucine (1 g) was dissolved in EtOH (5 ml), made weakly alkaline by adding 1 M ethanolic-KOH. After 4 days with an excess of methyl iodide, the soln was acidified to pH 1 with 6 N HCl and concentrated under vac. The remaining syrup was extracted with *n*-BuOH and the extract concentrated. The residue was dissolved in H_2O and shaken with Ag_2O. After filtration, the soln was concentrated under vac. The 60 MHz PMR spectrum of the remaining oil was identical with that obtained from the L-(+)-isoleucine betaine, synthesized by route A.

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