fractions were further fractionated by solvent extraction[3] and the solutions obtained purified by column chromatography, PLC and recrystallization Ajmalicine, ajmaline rescinnamine, reserpine serpentine and yohimbine were identified by co-TLC (4 systems) chromogenic reactions, fluorescence colours m p, m m p, UV IR and MS

Norajmaline base, amorphous yellow grey powder, acetate m p 165–170°, UV λ_{max} 244 289 nm IR ν_{max}^{KBr} 3350, 2950, 1715 1580 cm^{-1} MS *m/e* 312 (M^+) 297 283 186 182 169 168 144 143 131 130 (agrees with published data[4])

The principal alkaloids of the root are the dihydroindole bases ajmaline and norajmaline and the weak indole bases ajmalicine and reserpine

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ZEATIN IN *CANNABIS* FRUIT*

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Young fruits contain notable amounts of cytokinins which in maize, plums and apple fruits have proved to be zeatin or its derivatives [−8] while dihydrozeatin has been found in lupin seeds [9] These hormones play a role in the growth of fruits but may also be important in the development of the whole plant The different development of male and female individuals of dioecious hemp is most striking[1] and therefore, we were interested to characterize the cytokinins in hemp plants

The water fraction from a anionic ion exchange column showed considerable biological activity in the tobacco assay After chromatographic separation, two compounds were isolated, *A* and *B*, both of which possessed biological activity Their chromatographic behaviour was identical with zeatin and zeatin nucleoside respectively The compounds show the following spectral characteristics Compound *A* λEtOH 270 nm (max), 236 nm (min), λ 0 1 N HCl 273 nm (max), 237 nm (min), λ 0 1 N NaOH 221, 274 nm (max) with slight shoulder at 281–284 nm, 243 nm (min) Compound *B* λEtOH 276 nm (max)

* For Part I see Ref 1

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The UV-spectra of *A* in different solvents thus show absorption maxima comparable with both synthetic and natural zeatin and their identity was confirmed by GLC [3,10–13]

The absorption maximum in UV of *B* differs a little from that given for both the synthetic nucleoside[11] and that isolated from plants [4,12] Due to the low quantity of this compound, further purification and characterization was impossible but we suggest that it is identical with zeatin nucleoside

Assuming the purified cytokinins have molar extinction coefficients equal to that of zeatin and zeatin nucleoside, the yields of *A* and *B* are about 1 3 and 0 1 μmol respectively per kg of young hemp fruits

All the acid fractions from the column were also investigated chromatographically and only in 0 02 NHCl fraction bioassay of the zone with the R_f of zeatin nucleotide[5,14] showed activity in the tobacco callus assay Although the amount of this substance was very low its chromatographic properties suggest that indeed it is zeatin nucleotide which has been found in other fruits [5,8]

EXPERIMENTAL

Plant material Unripe fruits of hemp (*Cannabis sativa* L) were harvested in September 1972

Chemicals Zeatin was synthesized according to Shaw *et al*[11] by Dr D Munsche * Zeatin nucleoside was received from Dr D S Letham †

Chromatography For TLC silica gel HF_{254} was used, and for ascending PC Schleicher and Schull No 2043b paper

Chromatographic solvents Solvent A—*n*-BuOH–25% NH_4OH (4 1), Solvent B—water saturated *n*-BuOH on 0 03 M pH 8 4 borate impregnated paper,[14] Solvent C—*n*-BuOH–MeCOOH–H_2O (12 3 5),[14] Solvent D—MeOH–HCOOH–H_2O (16 3 1) The zones of chromatograms were eluted with 70% EtOH which was evaporated *in vacuo* at 35 GLC was done according to Upper *et al*[13] with some modification on a glass column (1 5 m × 4 mm) containing Gas Chrom Q (100–120 mesh) coated with 3% QF 1, N_2 (70 ml/min) and FID were used with a temp program 100–150°/4° (min)

The tobacco callus bioassay Pith callus tissue of tobacco (*Nicotiana tabacum* var Wisconsin No 38) was used The medium was that of Linsmaier and Skoog [15]

Extraction and separation of biological active compounds Young fruits of hemp (750 g) were homogenized with 80% MeOH and stored at 4 The supernatant after centrifugation was concentrated *in vacuo* at 35–40° to *ca* 0 3 vol The resulting solution was adjusted to pH 2 7 and percolated through columns of the cation-exchange resin "Wofatit-KPS" After washing, the columns were eluted with 4 N NH_4OH The NH_3 eluates were evaporated *in vacuo* at 35–40°, and the resulting sirup was dissolved in H_2O and transferred to four columns of Dowex 1 × 8 (Cl^-), 100–200 mesh, of 50 ml vol (1 8 × 20 cm) Each column was eluted with 1500 ml H_2O and subsequently with 500 ml portions 0 02 N HCl, 0 1 N HCl, 1 N HCl, 2 N HCl, and finally with 200 ml 4 N HCl The H_2O fraction was evaporated *in vacuo* Fractions containing acids were adjusted to pH 2 7 and passed through Dowex 50W × 8 (H^+) columns (50–100 mesh) The columns were washed thoroughly with H_2O and eluted with 4 N NH_4OH, which was evaporated *in vacuo* The residue of the water fraction was co-chromatographed with zeatin and zeatin nucleoside standards by TLC with solvent A The chromatographic zones indicated by these standards were eluted The substances obtained were purified 3 × by PC, using the solvents B, C and A The compounds corresponding to standards were eluted The residues from NH_4OH eluates (from "HCl fractions") were separated by PC 3 × with solvents C, D and C Only 0 02 N HCl and 0 1 N HCl fractions were first purified by TLC with solvent A The zones which might contain zeatin nucleotide according to[5,14] were eluted After the last chromatographic procedure the biological activities of the compounds or of the eluates were tested by tobacco callus assay and the UV-spectra were made

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