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SHORT REPORTS

3-NITRO-4-HYDROXY-PHENETHYLAMINE FROM *CEREUS VALIDUS*

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Key Word Index—*Cereus validus*; Cactaceae; 3-nitrotyramine; aromatic nitro compounds.

INTRODUCTION

In continuing our studies of Cactaceae occurring in the central west provinces of Argentina [1] we encountered a previously unstudied species of the genus *Cereus* which gave positive tests for alkaloids. Previous investigations of the genus *Cereus* have resulted in the isolation and identification of tyramine, hordenine and candicine [1–3]. We now report on the isolation, structure determination and synthesis of 3-nitro-4-hydroxyphenethylamine, one of the alkaloids of the cactus mentioned above, *C. validus*.

RESULTS AND DISCUSSION

The EtOH extract of fresh aerial parts of the plant gave a residue having a strong positive reaction for alkaloids. Residue was re-extracted and purified by chromatography yielding a crystalline yellow compound, pure by TLC, to which the 3-nitro-4-hydroxy-phenethylammonium hydroxide structure was assigned from the following evidence. Its UV spectrum showed maxima at λ 214 (ε 16000), 277 (6000) and 355 nm (2700), resembling those of a *o*-nitrohydroxy benzene system [4]. The IR spectrum showed bands characteristic of a nitro aromatic compound at 1530 and 1340 cm^{-1} together with those from phenyl, a monosubstituted ammonium salt, and asymmetric substitution in a benzene ring. The PMR spectrum showed signals of an AA'BB' system at δ 3.08 and 3.30 assigned to a dimethylenic chain such as the one present in tyramine hydrochloride [5] plus three aromatic protons at δ 7.15 (*d*, J = 8 Hz), 7.59 (*dd*, J = 8, J = 2 Hz), and 8.04 (*d*, J = 2 Hz) indicating an ABC system from a 1,2,4-trisubstituted aromatic ring. The MS did not show a M^+ but a M-18 peak at *m/e* 182 was present, and the fragmentation was typical of an aliphatic primary amine (*m/e* 30, base peak) and also to that of an aromatic nitrohydroxy compound (*m/e* 152, 135, 105, 83).

Our compound could be converted into a nitrate and into a hydrochloride and these salts were compared with synthetic 3-nitro-4-hydroxy-phenethylammonium nitrate and hydrochloride prepared from tyramine by a described procedure [6]. The compounds, natural and synthetic, were identical.

The common occurrence of phenethylamines in cacti is well documented [2] and is also valid for our plant. However, as far as we know, no nitrophenethylamine has been so far reported in the literature as a natural product. Previously known nitro compounds present in higher plants included aristolochic acids, nitro aliphatic, nitrogentisic acid, etc. and they have been subjected to numerous papers and reviews [7–12].

EXPERIMENTAL

Mp's were determined on a Kofler block and are uncorrected. TLC was performed on Si gel G. UV spectra were recorded in H_2O, IR in Nujol, PMR at 60 MHz in HOAc a-d_4 relative to TMS and MS at 70 eV with an ion source temp of 100°. Microanalysis was performed by B. B. de Deferrari (Universidad de Buenos Aires).

Plant material. C. validus was collected in the province of La Rioja (Argentina) in March (with fruits) and in October (without fruits). Dried in oven at 100–105° they lost respectively 92.6 and 88.1% of the original w. Voucher specimens have been deposited in the Colección de Cactaceas del Jardín Botánico de la Facultad de Ciencias Agrarias de la Universidad Nacional de Cuyo under No. 62.

Extraction of plant and isolation procedure. Fresh aerial parts (20 kg) with thorns and fruits removed were blended with EtOH (10 l.) and the mixture was left for 7 days. The extract obtained by filtration (24 l.) was concentrated *in vacuo* with the aid of n-BuOH, to 6 l. An aliquot (1.5 l.) corresponding to 370 g of dry plant material, was diluted with EtOH (4 l.) and the gummy dark ppt. (15.4 g) was filtered off and discarded (negative alkaloid test). The filtrate was concentrated *in vacuo* to a dark residue (36 g) having a strong positive test for alkaloids (Dragendorff–Munier). This residue was extracted with 2% HCl until it gave a negative alkaloid reaction, and the acidic extract was evaporated to dryness. The residue was taken up in MeOH (100 ml), a white insoluble ppt. (9.8 g) with no alkaloid reaction was filtered off, and the filtrate evaporated to dryness giving a residue (23.9 g) containing the 'raw alkaloid mixture'. This residue was dissolved in MeOH, a little Al_2O_3 added to the sol and the solvent evaporated. The Al_2O_3 which had adsorbed the extract was placed on the top of an Al_2O_3 (neutral, grade II–III) column (300 g) and eluted with $CHCl_3$–MeOH (100:0 to 72·5:27.5). The alkaloid-positive fractions ($CHCl_3$–MeOH 82.5:17.5 to 72.5:27.5) were

combined and evaporated leaving a yellow solid. Recrystallization from EtOH afforded an orange–yellow crystalline compound (689 mg; 0.19% of dry plant material). This was homogeneous on TLC in three different systems, $CHCl_3$–EtOH–HOAc (19:9:1) $CHCl_3$–MeOH (17:3), and EtOH–2N HCl (9:1). The isolated compound had mp 205–206°; UV (H_2O at pH 3): λ_{max} 214 (ε 16000), 277 (6000), 355 nm (2700); IR: 2700–2500 (several, $-{}^+NH_3$), 1610, 1565, 1500 ($-{}^+NH_3$), 1535, 1345 ($-NO_2$), 825, 805, 760 (trisubstituted benzene ring); PMR (HOAc-d_4:TMS): δ 3.08, 3.30 (AA'BB' system, Ph–CH_2–CH_2–$^+NH_3$), 7.15 (*d*, *J* = 8 Hz), 7.59 (*dd*, J = 8, *J* = 2 Hz), 8.04 (*d*, *J* = 2 Hz) (aromatic protons of a 1,2,4-trisubstituted benzene ring); MS: (*m/e*) 182 (M-18, 8%), 152 (182–30, 3%), 135 (182–HNO_2, 29%), 106 cf. [13] (182–30–NO_2, 11%), 105 (182–30–HNO_2, 16%), 83 (182–30–28–41, *o*-nitro effect, 16%), cf. [13] 77 (Ph^+, 20%), 51 ($C_4H_3^+$, 13%), 30 ($^+CH_2$–NH_2, 100%). Found: C, 47.86; H, 6.01; N, 13.90. $C_8H_{12}N_2O_4$ (MW 200.19) requires: C, 47.99; H, 6.04; N, 13.99%.

Preparation of the nitrate and the hydrochloride. The compound (40 mg) in H_2O (5 ml) was treated with conc HNO_3 (0.5 ml) and the soln evaporated to dryness. The residue was recrystallized from MeOH yielding the nitrate, mp 215–216° decomp. The compound (40 mg) was dissolved in boiling MeOH (15 ml) and treated with a few drops of conc HCl. The soln was evaporated and residue recrystallized from EtOH affording the hydrochloride, mp 214–215°.

Synthesis of 3-nitro-4-hydroxy-phenethylammonium nitrate. Tyramine (500 mg) was suspended in H_2O (3.5 ml) and treated at 0° with 56% HNO_3 (1.75 ml). The mixture was stirred at the same temp for 8 hr and then kept at 0° for 24 hr. The solid was filtered, washed with cold H_2O and MeOH, and dried. Recrystallization from MeOH gave 3-nitro-4-hydroxy-phenethylammonium nitrate (449 mg) mp 214–216° decomp (lit. [6] 217° decomp). The mp was not depressed on admixture with the nitrate from the natural compound.

Synthesis of 3-nitro-4-hydroxy-phenethylammonium hydrochloride. The synthetic product (100 mg) was dissolved in boiling MeOH, the soln treated with a few drops of conc HCl, and the whole evaporated to dryness. Recrystallization of the residue from EtOH afforded 3-nitro-4-hydroxy-phenethylammonium hydrochloride mp 213–214° (lit. [6] 214.5°). The mp was not depressed on admixture with the hydrochloride from the natural compound.

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HOMOSPERMIDINE IN *RHIZOBIUM* AND LEGUME ROOT NODULES

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Key Word Index—*Lupinus*; *Phaseolus*; *Pisum*; *Vicia*; Leguminosae; *Rhizobium*; lupin, pea and bean root nodules; homospermidine; polyamines.

I wish to report the identification of *sym*-homospermidine ($NH_2(CH_2)_4NH(CH_2)_4NH_2$) in legume root nodules and in the bacterial genus *Rhizobium*. This triamine, which is closely related structurally to the widely occurring polyamine, spermidine, has previously only been found in the free state in the leaves of the sandalwood tree (*Santalum album* L.) in which it comprises 0.5–1.5% of the dry weight [1–3]. In *Solanum tripartitum* homospermidine occurs as amide conjugates with fatty acids which have tumour inhibitory properties [4]. Other closely related polyamines occur rarely. *N*-3-Aminopropyl-1,5-diaminopentane has been found in a mutant of *Escherichia coli* [5]. Diaminodipropylamine ($NH_2(CH_2)_3NH(CH_2)_3NH_2$) has been reported in turnip yellow mosaic virus [6, 7], though this was subsequently disputed [8].

In the present work, using the dansyl technique, homospermidine has been demonstrated in root nodules of lupin, broad bean, runner bean, and pea, though it could not be found in the nodule-free roots of pea and broad bean (Table 1). Identification is based on TLC R_f, GLC R_t, and MS. The amine was detected by dansylation and TLC in the symbiotic nitrogen fixing bacteria, *Rhizobium* spp (Rothamsted strain 1045, *R. leguminosarum*, host *Pisum* and in strain 3824, *R. phaseoli*, host *Phaseolus*) grown on agar plates for 11 and 17 days resp. at 25°. Neither putrescine, spermidine, nor spermine could be detected in the bacteria. No polyamines were found in the agar gel. This is the first record of *sym*-homospermidine in bacteria.

Ions at *m/e* 168 in the MS of the TFA derivatives of spermidine and homospermidine indicate the presence of $—(CH_2)_4NH_2$ in both amines. Ions at *m/e* 154 ($—(CH_2)_3NHCOCF_3$) are present in TFA-spermidine