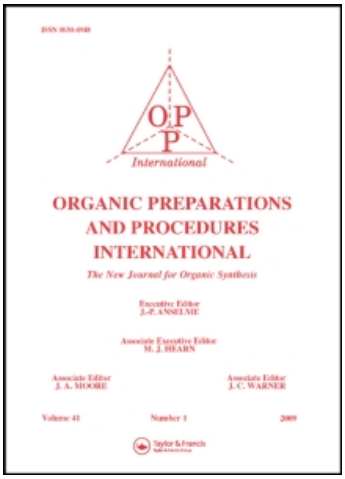

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SYNTHESIS OF NOPALINE AND ISO-NOPALINE

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SYNTHESIS OF NOPALINE AND ISO-NOPALINE

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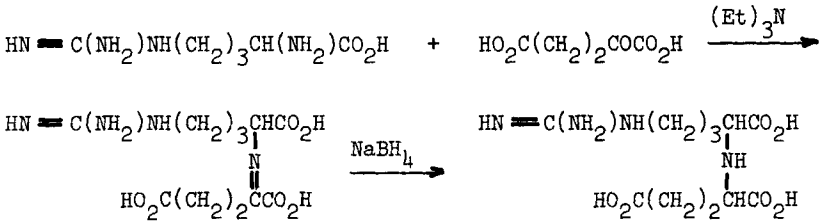
The unusual non-protein amino acid nopaline [N^2-(1,3-dicarboxypropyl)-arginine] occurs, often as the major free amino acid, in plant tumors (crown galls) induced by certain strains of Agrobacterium tumefaciens.[1] Thus far, studies on nopaline metabolism have relied on isolation of the naturally occurring compound from tumor tissue. The simple chemical synthesis for nopaline and its diastereoisomer iso-nopaline described here, has been achieved by the base catalysed condensation of 2-oxoglutaric acid with L-arginine, followed by borohydride reduction of the resultant Schiff's base.



EXPERIMENTAL

Nopaline and iso-nopaline.- Liquid reagents were dried over molecular sieve ($\frac{1}{8}$" pellets) and solids were finely ground and dried in vacuo over silica gel. 2-Oxoglutaric acid (10 g) dissolved in formamide (40 ml) was mixed with L-arginine (7 g, free base) suspended in formamide (40 ml) and the mixture was shaken until all solids had dissolved. After addition of triethylamine (30 ml), the mixture was left at 20° for 1.5 hour, before portionwise addition of sodium borohydride (5 g) suspended in ethanol (40 ml), over a period of 30 minutes. The reaction flask was cooled (5°) during addition of the borohydride and the mixture was then left standing for

at least 12 hours to allow excess borohydride to decompose. After dilution of the reaction mixture with formamide (70 ml), the crude nopaline isomers were precipitated by addition of pyridine (600 ml) with stirring. The sticky precipitate, recovered by filtration, was redissolved in formamide (150 ml), and then re-precipitated with pyridine (600 ml). The product was dissolved in water (200 ml) and evaporated at 40° to yield a thick syrup, which was finally evaporated with methanol (3 x 50 ml) to remove residual pyridine.

The crude product in water (200 ml) was passed through a column (42 cm x 3 cm) of Dowex 1 x 8, 20-50 mesh, $^{-}$OH form. The resin was washed with water (3.5 l), then eluted with 2 M acetic acid (9 ml/minute). Fractions (150 ml) were collected and those giving a strong Sakaguchi reaction,[2] usually fractions 2-5, were combined and evaporated at 50°. The resulting white solid was evaporated with ethanol (5 x 50 ml) to remove residual acetic acid, suspended in water (40 ml), filtered and dried *in vacuo* over silica gel to yield a white solid (4.6 g). This product was dissolved in boiling water (ca. 90 mg/ml) and left at 4° for 48 hours. Nopaline precipitated and was recovered by filtration; *iso*-nopaline was then precipitated from the filtrate by addition of ca. 20 volumes of ethanol. Both isomers were homogeneous on chromatography and electrophoresis[3] and contained less than 0.5% ninhydrin or Sakaguchi positive impurities. Permanganate oxidation[3] of both isomers gave arginine, γ-guanidobutyric acid and glutamic acid.

Nopaline.- Further recrystallization from water yielded nopaline (1.9 g) as clusters of fine needles, mp. 191° (uncorrected); $[\alpha]_D^{26°}$ + 12.5 ± 1.0° (c = 0.5 in water); solubility in water 9 g/l at 20°. Guanidine content calculated 19.1%, found 21.0% ± 2.5%. Low resolution MS (70eV) of the trimethyl ester-pyrazine derivative[4] showed major ions at m/e 123, 136 (base), 150, 151, 176, 205.

Anal. Calcd for $C_{11}H_{20}N_4O_6$: C, 43.41; H, 6.63; N, 18.41

Found: C, 43.59; H, 6.69; N, 18.06

TLC (160 μm thick cellulose): n-Butanol-acetic acid-water (4:1:1 by vol) (system I) R_f 0.14 ± 0.02; Pyridine-iso-amyl alcohol-water (8:4:7 by vol) (system II) R_f 0.61 ± 0.04.

Iso-nopaline (2.1 g) was recrystallized from water-ethanol and was obtained as individual microcrystals, mp. 164° (uncorrected); $[\alpha]_D^{26°}$ + 25.0 ± 2.0° (c = 0.5 in water); solubility in water ca. 70 g/l at 20°.

Anal. Calcd for $C_{11}H_{20}N_4O_6$: C, 43.41; H, 6.63; N, 18.41

Found: C, 43.20; H, 6.68; N, 18.36

R_f 0.15 ± 0.02 (system I); R_f 0.60 ± 0.04 (system II). Other data as for nopaline.

Natural nopaline; mp. 195° (uncorrected); $[\alpha]_D^{26°}$ + 11.8 ± 1.0° (c = 0.5 in water) was chromatographically and electrophoretically[3] identical with synthetic nopaline and was clearly different from iso-nopaline. Oxidation products and MS were also identical with those of the synthetic material.

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REFERENCES

† Deceased

1. A. Goldmann, D. W. Thomas and G. Morel, C. R. Acad. Sci., Ser. D. 268, 852 (1969).
2. J. B. Jepson and I. Smith, Nature, 172, 1100 (1953).
3. J. L. Firmin and R. G. Fenwick, Phytochemistry, in press (1977).
4. H. R. Morris, R. J. Dickinson and D. H. Williams, Biochem. Biophys. Res. Commun., 51, 247 (1973).