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BIOSYNTHESIS OF *N*-METHYL-5-CARBOXAMIDE-2-PYRIDONE FROM *TREWIA NUDIFLORA**†

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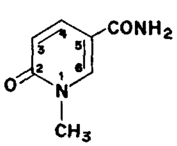
Key Word Index—*Trewia nudiflora*; Euphorbiaceae; *N*-methyl 5-carboxamide-2-pyridone; biosynthesis.

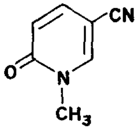
Abstract—A new plant constituent, *N*-methyl-5-carboxamide-2-pyridone (I) has been isolated and identified from *Trewia nudiflora*. This compound is a normal metabolite of nicotinic acid in mammals and has not previously been identified in plants. Biosynthesis of *N*-methyl-5-carboxamide-2-pyridone from nicotinic acid(-6-^{14}C or -7-^{14}C) and nicotinamide-7-^{14}C was established.

INTRODUCTION

Trewia nudiflora, a tropical plant native to India, contains the alkaloid nudiflorine (II, *N*-methyl-5-cyano-2-pyridone) whose structure was proven by Mukherjee and Chatterjee[1] and ricinidine (III, *N*-methyl-3-cyano-2-pyridone) whose structure was recently proven by Ganguli.[2] It was found that young *T. nudiflora* plants grown from seeds in either the green house or in field plots contained only a trace of nudiflorine; however, other pyridine alkaloids were detected. The pyridone alkaloids were isotropically labeled with ^{14}C by injecting nicotinic acid-6-^{14}C or -7-^{14}C into the plants and isolating the radioactive alkaloid fraction. Six alkaloids were isolated from the plant extract. Of special interest was the discovery of



(I)



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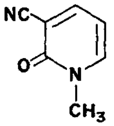

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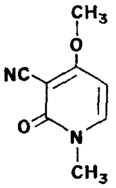

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† For preliminary communications see Waller, G. R. and Sastry, S. D., Abstracts, American Chemical Society, 158th National Meeting, New York, (1969). BIOL. 133.

[1] R. MUKHERJEE and A. CHATTERJEE, *Tetrahedron* **22,** 1461 (1966).

[2] S. N. GANGULI, *Phytochem.* **9,** 1667 (1970).

N-methyl-5-carboxamide-2-pyridone (I), a normal metabolite of nicotinic acid in mammals, which had not been previously isolated from plants. With the exception of trigonelline (*N*-methyl nicotinic acid), a widely occurring pyridine compound in the plant kingdom, the other alkaloids remain unidentified.

RESULTS

Identification of Pyridine Compounds

N-*Methyl-5-carboxymide-2-pyridone.* The major component of the methanol fraction was isolated by preparative TLC. The compound was further purified by several preparative TLC runs using methanol, chloroform (1:50 and 2:5 consecutively) to a white crystalline solid. The UV spectrum in methanol had an absorption at 258 nm due to the pyridone functional group. Its IR spectrum (KBr) showed bands at 3420, 1660 and 1600 cm^{-1} due to a conjugated amide group. Mass spectral analysis of this compound showed it to have a MW 152. The MS also showed intense fragment ions at *m/e* [M$^+$-16(–NH$_2$)] and *m/e* 108 [M$^+$-44(–CONH$_2$)]. Identity of the compound was confirmed by TLC with authentic samples of *N*-methyl-5-carboxamide-2-pyridone and *N*-methyl-3-carboxamide-2-pyridone. M.m.p. with *N*-methyl-5-carboxamide-2-pyridone did not show any depression.

Nudiflorine. This alkaloid was present only in trace amounts in young plants. Nudiflorine moved to the solvent front in the chloroform–methanol (9:1) solvent system and a trace of radioactivity was found at this R_f. The collected solvent front band of several TLC fractions were extracted with methanol. The substance was rechromatographed in butanol–formic acid–water (12:1·7). A very small amount of a compound with similar R_f value as standard nudiflorine was isolated. The UV absorptions at λ_{max} 207, 252, 303 nm were similar to those of the nudiflorine spectrum. An accurate specific radioactivity was not obtained.

N-*methyl-nicotinic acid.* The aqueous extract on TLC in 2-propanol-water (7:3) solvent system showed three compounds at R_f values of 0·8, 0·62 and 0·38. Preparative TLC of the minor spot yielded a small amount of compound that had an UV spectral absorption at λ_{max} 261 due to the pyridine moiety. The MS (direct probe) of this compound showed major fragment ions at *m/e* 138, *m/e* 94, *m/e* 93 and *m/e* 78 indicating the presence of an *N*-methyl nicotinic acid moiety. The fragment ion at *m/e* 138 is *N*-methyl nicotinic acid and other ions *m/e* 94 and *m/e* 93 are formed by loss of –CO$_2$ and –COOH, respectively, from *m/e* 138. The pyridine nucleus is *m/e* 78. All spectral and chromatographic data agreed with corresponding data obtained from authentic *N*-methyl nicotinic acid.

Biosynthesis

In a typical experiment, nicotinic acid-6-^{14}C, total radioactivity 1·3 × 10^6 dpm/mg, was dissolved in 0·2 ml distilled water and injected into a young *T. nudiflora* plant. After 48 hr the plant material was worked up as described. Upon subjecting the methanol extract to thin layer chromatography (ChromAR 1000) major fluorescent and radioactive zones, including the zone at the origin, were observed. The major compound with R_f 0·36 was identified as *N*-methyl-5-carboxamide-2-pyridone (I). Work to identify other compounds, which remain unknown, is in progress. Control experiments had the same fluoresecent zones.

When the aqueous extract from the biosynthesis experiments was subjected to TLC (ChromAR 1000), two radioactive peaks were observed. Sufficient quantity was available so that it was possible to isolate them by preparative thin layer chromatography. The compound with R_f 0·38 was identified as *N*-methyl-nicotinic acid by UV absorption, MS and chromatographic retention time. The radioactive band at R_f 0·8 was subjected to further TLC analysis using a chloroform–methanol (5:1) solvent system and several radioactive compounds could be separated. One of them was identified as *N*-methyl-5-carboxamide-2-pyridone which was a major component of the methanol fraction indicating that extraction with hot methanol was incomplete; however, only *ca* 10% of the total *N*-methyl-5-carboxamide-2-pyridone was isolated in this fraction.

Results from the incorporation of nicotinic acid-7-^{14}C and nicotinamide-7-^{14}C into *N*-methyl-5-carboxamide-2-pyridone in *T. nudiflora* conducted for 24, 48- and 136-hr time periods are presented in Table 1. After 6 days the dilution of nicotinic acid-7-^{14}C was 13-fold and the dilution of nicotinamide was 11·5 fold. Biosynthesis experiments using nicotinic acid-6-^{14}C produced similar dilution results to those shown in Table 1 for nicotinic acid-7-^{14}C. These small differences do not permit conclusions to be drawn as to the relative positions of these precursors in the biosynthetic pathway.

TABLE 1. FORMATION OF *N*-METHYL-5-CARBOXAMIDE-2-PYRIDONE FROM NICOTINIC ACID-7-^{14}C AND NICOTINAMIDE-7-^{14}C

Precursor*	Time of sampling (hr)	Activity of precursor: Specific activity (dpm/mg × 10^6)	Activity of precursor: Total activity used (dpm × 10^6)	Specific activity of *N*-methyl-5-carboxamide-2-pyridone (dpm/mg × 10^4)	Isotope dilution
Nicotinic acid-7-^{14}C	24	4·1	3·6	4·1	100
	48	4·1	2·2	5·1	80·4
	136	4·1	2·1	31·0	13·2
Nicotinamide-7-^{14}C	136	2·2	2·2	19·0	11·5

* Labelled compounds were administered to the whole plant.

DISCUSSION

Conclusive evidence for the identification of a new pyridine alkaloid, *N*-methyl-5-carboxamide-2-pyridone, from *Trewia nudiflora* and its biosynthesis from nicotinic acid-6-^{14}C, -7-^{14}C and nicotinamide-7-^{14}C was established. Previous work from this laboratory established the existence of a pyridine nucleotide cycle in two alkaloid-producing plants and it was shown that the pyridinum moiety of each member of the cycle could give rise to the pyridine ring of ricinine[3] and nicotine.[4] It seems reasonable to assume that this cycle occurs ubiquitously.[5] Consequently we suggest that a similar nicotinic acid and nicotinamide metabolic pathway exists in *T. nudiflora.* A plausible biogenetic scheme for the formation of *N*-methyl-5-carboxamide-2-pyridone is shown in Fig. 1. *N*-methyl nicotinamide is formed from nicotinamide. Carbon-2 of *N*-methylnicotinamide undergoes oxidation to give *N*-methyl-5-carboxamide-2-pyridone. The mechanism involved in the oxidation remains unknown.

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[3] G. R. WALLER, K. S. YANG, R. K. GHOLSON, L. A. HADWIGER and S. CHAYKIN, *J. Biol. Chem.* **241,** 4411 (1966).

[4] G. M. FROST, K. S. YANG and G. R. WALLER, *J. Biol. Chem.* **242,** 887 (1967).

[5] R. K. GHOLSON, *Nature, Lond.* **212,** 934 (1966).

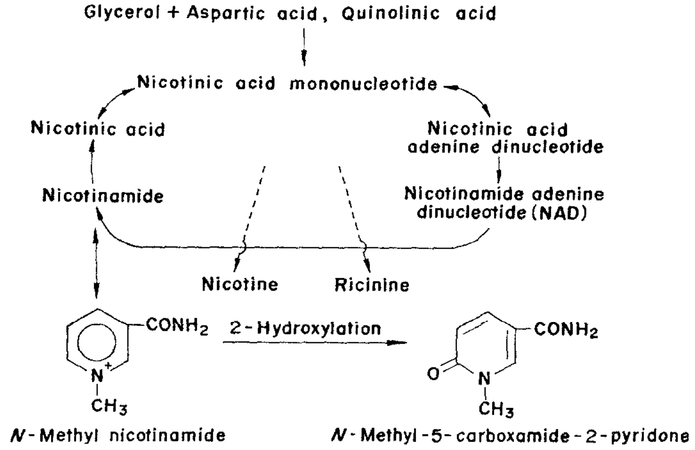

FIG. 1. BIOGENESIS OF *N*-METHYL-5-CARBOXAMIDE-2-PYRIDINE IN *T. nudiflora*

The identification of ricinidine (III)[2] from *T. nudiflora* indicates that this plant has a hydroxylation system capable of acting on both carbons 2 and 6 (in contrast, in *Ricinus communis* which produces ricinine (IV), only C-6 and C-4 hydroxylation occur). This lends support to Mukherjee's and Chatterjee's hypothesis[1] that oxidation at carbons 2 and 6 occurs in *N*-methyl nicotinamide to yield the hydroxylated compounds which may serve as direct precursors of nudiflorine (II) or ricinidine (III). For nudiflorine (II) biosynthesis, C-2 hydroxylation probably occurs in *N*-methyl nicotinamide, which further forms *N*-methyl-5-carboxamide-2-pyridone (I). Intramolecular dehydration of the amide group of I gives II. For ricinidine (III) biosynthesis, C-6 hydroxylation probably occurs in *N*-methyl nicotinamide which could further form *N*-methyl-5-carboxamide-6-pyridone; the dehydration of the amide group would give III. We were unable to isolate *N*-methyl-5-carboxamide-6-pyridone or ricinidine (III) from the young *T. nudiflora* plants used in the experiments reported here. Biosynthesis of these 6-pyridone alkaloids likely occurs at a later stage in the life cycle.

EXPERIMENTAL

Labeled compounds. Nicotinic acid-6-^{14}C and nicotinamide-7-^{14}C were purchased from New England Nuclear Corporation, Boston, Massachusetts. Labeled nicotinic acid was purified by TLC using 2-propanol-water (85:15) as a solvent system and nicotinamide using methanol–chloroform (1:9). All compounds were more than 99% radiochemically pure.

Reference compounds. N-methyl-5-carboxamide-2-pyridone was a gift from Dr. Sterling Chaykin and nudiflorine was a gift from Dr. A. Chatterjee.

Plants. T. nudiflora plants from 1 to 2 yr of age grown from seed, obtained near Calcutta, India, in the greenhouse or at the Horticultural Farm near Stillwater, Oklahoma, were used.

Administration of labeled compounds. Nicotinic acid-6-^{14}C, nicotinic acid-7-^{14}C and nicotinamide-7-^{14}C were dissolved in distilled water and injected into the stem of whole *T. nudiflora* plants.

Isolation of alkaloids. Fresh plants were homogenized and extracted twice with chloroform (2 × 200 ml). The residue was further extracted with methanol–water (3:1; 5 × 200 ml) until the residue was free of soluble pigments. The chloroform and methanol–water extracts were combined and concentrated under reduced pressure to about 25 ml, cooled and the aqueous solution decanted. The residue was rinsed twice with water. The aqueous fraction was taken to dryness under reduced pressure. The residue was then extracted with hot methanol (5 × 50 ml). The collected methanol extract was concentrated and used for isolation of the alkaloids. The residue was then dissolved in water and used for isolation of alkaloids.

TLC. ChromAR sheet 1000 and 500 purchased from Malinckrodt Chemical Works were used for preparative and analytical TLC respectively. The solvent system used for the methanol soluble fraction was chloroform–methanol (9:1 v/v) and for the aqueous fraction, 2-propanol–water (7:3, v/v). Pyridine compounds were detected by observing their fluorescence at 256 and 366 nm.

Isotope analysis. The radioactivity on the chromatograms was located using a Nuclear Chicago 4-Pi Actigraph-III strip counter. The specific activity of the precursors and the alkaloids was determined using a Packard Tri-Carb Model 3320 liquid scintillation spectrometer.

Spectra. UV spectra were obtained on a Bausch and Lomb Model 505 spectrophotometer. IR spectra were obtained with a Perkin–Elmer Model-457 grating instrument. MS were obtained on the LKB-9000 prototype[6] combination GLC–MS at 70 eV. The temperature of the direct inlet was 60° and of the ion source 310°.

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[6] G. R. Waller, *Proc. Okla. Acad. Sci.* **47**, 271 (1968).