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BIOSYNTHESIS OF PYRIDINE ALKALOIDS FROM *TRIPTERYGIUM WILFORDII**

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Key Word Index—*Tripterygium wilfordii*; Celastraceae; alkaloid biosynthesis; pyridine alkaloids; pyridine nucleotide cycle.

Abstract—Nicotinic acid-6-^{14}C and nicotinamide adenine dinucleotide-carbonyl-^{14}C were rapidly metabolized in *T. wilfordii* Hook. with formation of all compounds in the pyridine nucleotide cycle. Nicotinic acid-6-^{14}C and the nicotinamide moiety of NAD were efficiently incorporated into wilfordic acid and hydroxywilfordic acid, the pyridinium moieties of the ester alkaloids. The structures of wilfordic acid and hydroxywilfordic acid were confirmed using GLC-MS. The molecular formulae of the four isolated alkaloids were determined by high resolution MS and agreed with earlier results based on elemental analysis.

INTRODUCTION

A NATURALLY occurring insecticidal material non-toxic to warm blooded animals was discovered in the early 1950s.[1-3] The insecticidal material is contained in *Tripterygium wilfordii* Hook. (Celastraceae), a plant known as the 'Thunder God Vine' in its native Chinese habitat. Its toxic qualities come from the root alkaloids which have been used for centuries by Chinese gardeners to protect their crops against chewing insects. Four of these alkaloids have been isolated: wilforine, wilforgine, wilfordine and wilfortrine. Upon alkaline saponification, each of the complex ester alkaloids yields a derivative of nicotinic acid, either wilfordic acid (I) or hydroxywilfordic acid (II).[4] The complete structures of these ester alkaloids are not yet known; however, the structures of the pyridinium components, wilfordic acid and hydroxywilfordic acid, have been confirmed using mass spectral techniques and the results are described herein.

The purpose of this research was to determine if nicotinic acid and nicotinamide adenine dinucleotide, obligatory members of the pyridine nucleotide cycle,[5] could serve as precursors

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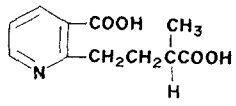
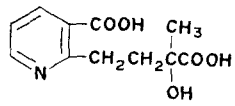
† This research was done in partial fulfilment of the requirements for the degree of Ph.D. at Oklahoma State University. Present address: Research Institute for Skeletomuscular Diseases, Mount Sinai School of Medicine, NY 10035, U.S.A.

[1] M. BEROZA, *J. Am. Chem. Soc.* **73**, 3656 (1951).
[2] M. BEROZA, *J. Am. Chem. Soc.* **74**, 1585 (1952).
[3] M. BEROZA, *J. Am. Chem. Soc.* **75**, 44 (1952).
[4] M. BEROZA, *J. Org. Chem.* **28**, 3562 (1963).
[5] G. R. WALLER, K. S. YANG, R. K. GHOLSON, L. A. HADWIGER and S. CHAYKIN, *J. Biol. Chem.* **241**, 4411 (1966).

of the pyridine moiety of the *Tripterygium wilfordii* alkaloids. Positive results from such a study would serve to confirm and extend the pyridine nucleotide cycle -pyridine alkaloid inter-relationships.



Wilfordic acid (I)



Hydroxywilfordic acid (II)

RESULTS AND DISCUSSION

Isolation and Identification of Alkaloids and of their Pyridinium Components

Attempts to separate the four structurally related alkaloids from the alkaloid fraction by TLC were unsuccessful. Therefore, the alkaloid zone on a thin layer plate developed with acetone–hexane (4:1) was scraped off, extracted, crystallized and the mixture of crystallized alkaloids was subjected to partition column chromatography on silicic acid using hydrochloric acid as immobile solvent and ether as mobile solvent.[6] Tubes from the peaks of interest were pooled, subjected to TLC, recrystallized from acetone and methanol and identified mass spectrometrically.

The MS of wilforine, wilforgine, wilfordine and wilfortrine showed that their molecular ions were identical with the reported MWs based on elemental analyses.[1,2] The MW and elemental formulae of the four ester alkaloids, wilforine (MW 867, $C_{43}H_{49}O_{18}N$), wilforgine (MW 857, $C_{41}H_{47}O_{19}N$), wilfordine (MW 883, $C_{43}H_{49}O_{19}N$) and wilfortrine (MW 873, $C_{41}H_{47}O_{20}N$) were determined using low and high resolution MS.[7]

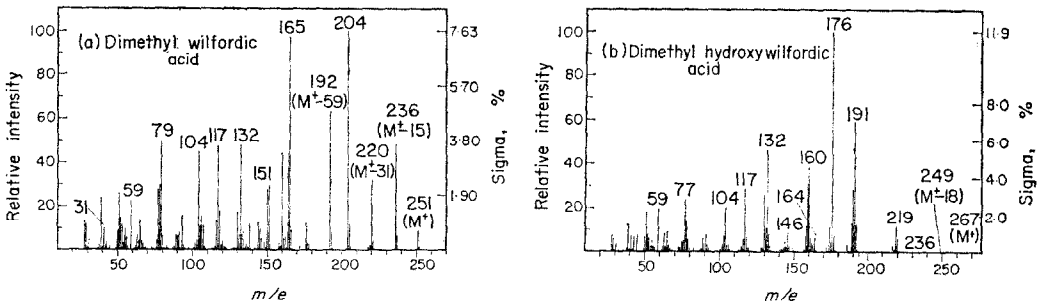


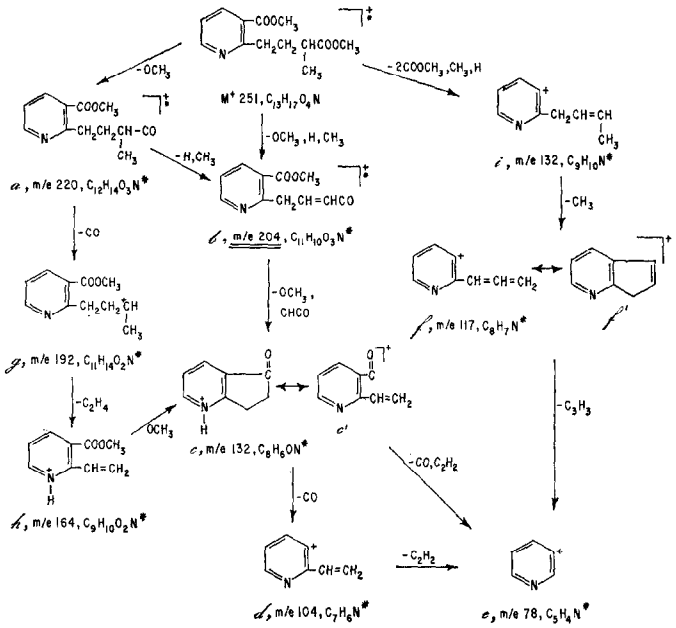
FIG. 1. MASS SPECTRA OF DIMETHYL WILFORDIC ACID (a) AND DIMETHYL HYDROXYWILFORDIC ACID (b). LKB-9000 (15); 70 eV ionization voltage; 3·5 kV accelerating voltage; 310° source temp.; 250° separator temp. GLC conditions were: 7 ft × ⅛ in. glass column packed with 3% SE-30 on Gas Chrom Q, 100–120 mesh; 190° column temp. and 215° injector temp.

The isolated alkaloid fraction was hydrolyzed with 1 M KOH in diethylene–glycol and fractionated by liquid–liquid extraction;[2] the acidic components were methylated with diazomethane and analyzed by GLC–MS. Four components were identified: wilfordate, hydroxywilfordate, benzoate and acetate. The presence of multiple hydroxy groups in the

[6] M. BEROZA, *Analyt. Chem.* **22**, 1507 (1950).
[7] H. LEE, Ph.D. Dissertation, Oklahoma State University, Stillwater, Oklahoma (1971).

neutral polyhydroxy fraction was proved by obtaining NMR spectra on the deuterium exchanged compound.[7]

The MS of dimethyl wilfordic acid is shown in Fig. 1(a). The molecular ion, M^+ 251, corresponds to the MW of the dimethyl ester of wilfordic acid. The spectrum exhibited a characteristic fragmentation pattern of a pyridine compound with an α-side chain.[8] The



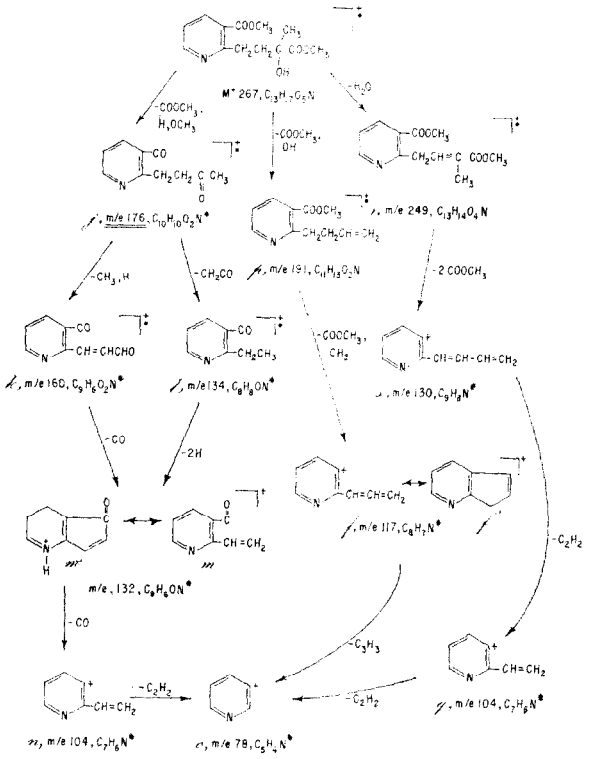
SCHEME 1. PROPOSED PARTIAL FRAGMENTATION OF DIMETHYLWILFORDATE.
*Molecular formulae were determined on high resolution MS of ester alkaloid.

proposed fragmentation of dimethyl wilfordic acid is shown in Scheme 1. The molecular ion M^+ 251, $C_{13}H_{17}O_4N$, loses a methoxy group,[9] to form the fragment ion *a*, *m/e* 220, $C_{12}H_{14}O_3N$. The base peak *b* is formed either from *a*, by loss of one hydrogen atom and a methyl group or directly from the molecular ion by the successive loss of the neutral fragments CH_3, H and OCH_3. Ion *b* loses OCH_3 and CHCO to form the ion *c*, *m/e* 132, C_8H_6 ON; further stepwise loss of CO and C_2H_2 can occur to form ions *d* C_7H_6N, and *e*, C_5H_4N, respectively. Ion *e*, which is a common fragment formed from substituted pyridine compounds is formed either from the ion *c*, by loss of CO and C_2H_2 or from ion *f*, by the loss of C_3H_3. Ion *a* loses 28 mass units (CO) to yield ion *g*, $C_{11}H_{14}O_2N$, which loses C_2H_4 to form ions *h*, *m/e* 164, $C_9H_{10}O_2N$, which can give ion *c* by loss of OCH_3. Ion *i*, *m/e* 132, $C_9H_{10}N$, is formed from the molecular ion by loss of two $COOCH_3$ groups and a hydrogen atom. Further successive loss of CH_3 and C_3H_3 from *i* yields ions *f*, *m/e* 117, C_8H_7N, (which can rearrange to give *f'*) and *e*, C_5H_4N, respectively.

The MS of dimethyl hydroxy wilfordic acid is shown in Fig. 1(b). This spectrum does not show a peak corresponding to the molecular ion, M^+ 267; however, the ion *h*, *m/e* 249,

[8] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Interpretation of Mass Spectra of Organic Compounds*, pp. 225–257, Holden-Day, San Francisco (1965).
[9] E. GIL-AV, J. H. LEFTIN, A. MANDELBAUM and S. WEINSTEIN, *Org. Mass Spectros.* **4**, 475 (1970).

$C_{13}H_{15}O_4N$, formed by the loss of water [$M^+ - 18$ (H_2O)] from the molecular ion is present. The absence of the molecular ion due to elimination of water from higher alcohols is a common phenomenon;[10] in higher alcohols, dehydration may also occur as a result of thermal decomposition prior to electron impact, a finding confirmed here by observation that the intensity of the $M^+ - 18$ ion (*h*) was greatly reduced when the sample was inserted near the ion source.[11] The proposed fragmentation of dimethyl hydroxywilfordic acid is shown in Scheme 2. The base peak *j*, *m/e* 176, $C_{10}H_{10}O_2N$, is formed by the loss of a methoxyl group from the side chain at C-3 and $HCOOCH_3$ from the side chain at C-2. Ion *j* successively



SCHEME 2. PROPOSED PARTIAL FRAGMENTATION OF DIMETHYL HYDROXYWILFORDIC ACID.
*Molecular formulae were determined on high resolution MS of ester alkaloid.

loses the neutral fragments, CH_3 and H, or CH_2CO to form ions *k*, *m/e* 160, $C_9H_6O_2N$, and *l*, *m/e* 134, C_8H_8ON. The loss of carbon monoxide from *k* and two hydrogen atoms from ion *l* results in the formation of ion *m*, *m/e* 132, which is structurally stabilized (*m'*) due to the conjugation of the double bond with the aromatic pyridine ring. Loss of CO from ion *m* (or *m'*) gives ion *n* which loses acetylene to give ion *o*. The molecular ion, M^+ 267, also loses CH_3COO and a hydroxyl group from the C-2 side chain to form the fragment ion *p*, *m/e* 191, $C_{11}H_{13}O_2N$; further successive loss of $COOCH_3$, CH_3 and C_3H_3 occurs to form ion *f*, *m/e* 78, $C_6H_{14}N$ respectively. The ion *q*, *m/e* 104, C_7H_6N, is formed from ion *r*, *m/e*

[10] F. W. MCLAFFERTY, *Mass Spectrometry of Organic Ions*, p. 318, Academic Press, New York (1963).
[11] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Interpretation of Mass Spectra of Organic Compounds*, p. 33, Holden-Day, San Francisco (1965).

249, $C_{13}H_{15}O_4N$ by the successive loss of two $COOCH_3$ fragments to give ion *s* and *q* by the loss of acetylene from ion *s*. Ion *q* then loses acetylene to give *o*.

Biosynthesis of T. wilfordii *Alkaloids*

Isolation and identification of nicotinic acid and NAD metabolites. When a portion of the 80% methanol extract of the plant fed with nicotinic acid-6-^{14}C was subjected to TLC in acetone–hexane (4:1), two main radioactive zones, an alkaloid and a polar zone, were observed. More than 90% of the isolated radioactivity was located at the polar zone. The polar compounds were removed from the plate and subjected to Dowex 1-X8 formate anion exchange column chromatography. Figure 2 shows the results of the metabolism of nicotinic acid-6-^{14}C in the aerial parts (leaves and stems) and in the roots of *T. wilfordii* respectively. More than ten radioactive peaks were found. The height and number of the peaks (six main peaks) were smaller in the radiochromatogram of root extracts. Tubes representing each peak were pooled, lyophilized and identified by PC with authentic samples in two solvents. Based on R_f values, elution volumes on anion exchange chromatography and UV spectra, the radioactive peaks, N-1, N-2, N-7, N-8, N-11 and N-13 were tentatively identified as *N*-methylnicotinamide, nicotinic acid, NAD, NaMN and desamido-NAD respectively.

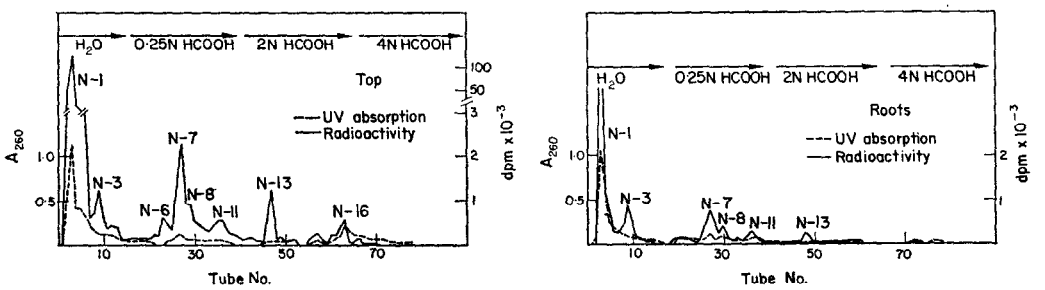


FIG. 2. DOWEX 1-X8 FORMATE COLUMN CHROMATOGRAPHY OF THE NA-6-^{14}C METABOLITES FROM *T. wilfordii*.

Column, 1·5 × 30 cm, was eluted by the application of a formic acid concentration gradient initially with 150 ml of water in the mixing chamber, into which 150 ml of deionized water, 250 ml of 0·25 N formic acid, 250 ml of 2 N formic acid and 250 ml of 4 N formic acid were introduced. Fractions of 10 ml were collected at a flow rate of 35 ml/hr. Upper and lower figures are the chromatograms of the metabolites from the top and the roots of the plant respectively. The solid and broken lines represent radioactivity and absorbancy at 260 nm respectively. The radioactivity peaks, N-1, N-2, N-7, N-8, N-11 and N-13 were tentatively identified as *N'*-methylnicotinamide, nicotinamide, nicotinic acid, NAD, NaMN and desamidoNAD respectively.

Nicotinic acid-6-^{14}C *and NAD-carbonyl-*^{14}C *as precursors.* Although the biosynthesis of the pyridine alkaloids, especially ricinine,[5] nicotine[12] and anabasine,[13] has been studied extensively, no attempt has been made to elucidate the origin of pyridine moieties of the *T. wilfordii* ester alkaloids, wilfordic acid and hydroxywilfordic acid. The results of feeding nicotinic acid-6-^{14}C and NAD-carbonyl-^{14}C to *T. wilfordii* plants for four days are shown in Table 1. The radioactivity of alkaloids in the roots was much higher than that in the leaves and stems when nicotinic acid-6-^{14}C was the precursor. Both precursors were injected into

[12] J. FLEEKER and R. U. BYERRUM, *J. Biol. Chem.* **240**, 4099 (1965).
[13] A. R. FRIEDMAN and E. LEETE, *J. Am. Chem. Soc.* **85**, 2141 (1963).

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN *T. wilfordii* AFTER ADMINISTRATION OF NICOTINIC ACID-6-^{14}C AND NAD-CARBONYL-^{14}C

Administered compound	Compound	Roots (dpm × 10^{-4})	(%)	Leaves and stems (dpm × 10^{-4})	(%)	Total (dpm × 10^{-4})	(%)
Nicotinic acid-6-^{14}C	Alkaloids	2·072	3·21	0·764	1·19	2·837	4·40
	Polar compounds	2·267	3·52	20·106	31·18	22·373	34·69
	Total	4·339	6·73	20·870	32·36	25·210	39·09
NAD-carbonyl-^{14}C	Alkaloids	0·242	0·5	0·777	1·4	1·019	2·3
	Polar compounds	1·486	3·4	16·686	37·6	18·172	41·0
	Total	1·728	3·9	17·463	39·4	19·191	43·3

Duration of experiment was 4 days and radioactivities were average of two experiments. The plants were grown in the green house. Nicotinic acid-6-^{14}C ($1·1 \times 10^{-2}$ μM) with a total radioactivity of $64·487 \times 10^4$ dpm and NAD-carbonyl-^{14}C (10^{-2} μM) with a total radioactivity of $44·353 \times 10^4$ dpm were administered. Percentage of incorporation was determined by dividing total radioactivity administered by the total amount recovered.

the stems. The total percentage of the incorporation of nicotinic acid-6-^{14}C into the alkaloids was about twice that of NAD-carbonyl-^{14}C. The difference in extent of total incorporation between nicotinic acid and NAD is due to their different efficiencies of incorporation into alkaloids in roots as compared to stems and leaves. The radioactivity in the alkaloids of the leaves and stems was actually slightly higher when NAD was fed than when nicotinic acid was fed (1·4 compared to 1·2%); however, the radioactivity of the alkaloids in the roots was much lower when NAD was administered than when nicotinic acid was administered (3·2 compared to 0·5%). The incorporation efficiencies of nicotinic acid and NAD into *T. wilfordii* alkaloids are comparable if it is assumed that the differences in the radioactivity of the alkaloids in the roots of the plants fed nicotinic acid and NAD were due to other factors such as site of synthesis of the alkaloids and translocation and/or uptake of the precursors.

Metabolism of nicotinic acid-6-^{14}C and NAD-carbonyl-^{14}C in the plant. During the biosynthesis of the alkaloids in *T. wilfordii*, 80–90% of the isolated radioactivity remained in the polar compounds after either extracting the alkaloids with organic solvent or TLC in a relatively non-polar solvent. To elucidate the relationship between the pyridine nucleotide cycle and alkaloid biosynthesis in *T. wilfordii*, a study of the polar compounds in aqueous phase was undertaken. As shown in Table 2, the total radioactivity recovered from the plant

TABLE 2. RADIOACTIVITY DISTRIBUTION AFTER ADMINISTRATION OF NICOTINIC ACID-6-^{14}C AND NAD-CARBONYL-^{14}C INTO *T. wilfordii* PLANTS

Administered compound / Plant part / Metabolite	Nicotinic acid-6-^{14}C Root	Top	Total (dpm × 10^{-3})	(%)	NAD-carbonyl-^{14}C Root	Top	Total (dpm × 10^{-3})	(%)
N'-methylnicotinamide	16·286	156·679	172·965	26·82	12·889	36·993	49·882	11·25
Nicotinamide	1·682	4·396	6·078	0·94	0·189	0·282	0·468	0·11
Nicotinic acid	1·753	8·739	10·492	1·63	1·219	57·425	57·763	13·02
NAD	0·839	2·270	3·109	0·48	0·119			
Nicotinic acid mononucleotide	0·593	1·330	1·923	0·30	0·081	26·775	26·861	6·06
Desamido-NAD	0·526	1·896	2·422	0·38	0·186	19·245	19·431	4·38
Alkaloids	20·732	7·648	28·371	4·40	2·426	7·765	10·200	2·29
Others*	0·931	27·249	28·237	4·38	1·269	26·160	27·323	6·16
Total	43·390	208·707	252·097	39·09	17·291	174·639	191·928	43·27

* Unclassified polar compounds. Average of two experiments. Duration of the experiment was 4 days. The plants were grown in the green house. Nicotinic acid-6-^{14}C ($1·1 \times 10^{-2}$ μM) with a total radioactivity of $64·484 \times 10^4$ dpm, and NAD-carbonyl^{14}C (10^{-2} μM) with a total radioactivity of $44·353 \times 10^4$ dpm was administered. Percentage of incorporation was determined by dividing the total radioactivity administered by the total amount recovered.

fed with NAD was higher than that of the plant fed with nicotinic acid; the distribution of radioactivity among the metabolites was also remarkably different .The ratio of radioactivity present in the roots compared to that in the aerial parts was higher when nicotinic acid was administered than when NAD was administered. This difference might reflect the difference between nicotinic acid and NAD with respect to uptake and/or translocation. The distribution of radioactivity among metabolites of nicotinic acid varied greatly. The incorporation percentage of nicotinic acid into the non-nucleotide compounds, especially *N*-methylnicotinamide, was much higher than in the nucleotides. The radioactivity distribution among nucleotides such as NAD, nicotinic acid mononucleotide and desamido-NAD was relatively uniform, ranging from 0·3 to 0·48% of the total administered radioactivity.

The distribution of radioactivity among the nucleotides such as NAD^+, nicotinic acid mononucleotide and desamido-NAD^+ was lower in the roots and much higher in the leaves and stems when NAD was fed than when nicotinic acid was fed. The fact that labelled NAD^+ gives rise to higher radioactivity in nicotinic acid mononucleotide and desamido-NAD in the leaves and stems than does nicotinic acid suggests that phosphoribosylpyrophosphate may be the rate limiting compound in the cycle. It is known that degradation of the pyridine nucleotides is the only biological source of nicotinamide and subsequently nicotinic acid, the immediate precursor for nicotinic acid mononucleotide.[14] Desamido-NAD is not formed from NAD. Therefore, the concentration of nucleotides in the pyridine nucleotide cycle might be governed by the concentration of phosphoribosylpyrophosphate (PRPP) since it is required for *de novo* and salvage synthesis of nicotinic acid mononucleotide. An alternate circuit loop of the main cycle (NAD → NMN → nicotinamide), found in yeast,[15] and also detected in barley,[16] cannot operate in *T. wilfordii*, since nicotinamide mononucleotide could not be detected.

It is well known that the biosynthesis of NAD^+ from nicotinic acid via nicotinic acid mononucleotide and desamido-NAD^+ occurs in higher plant families such as the Euphorbiaceae and Solanaceae, and certain bacteria.[17] The experiments reported here provide evidence for the function of this pathway in yet another family, the Celastraceae.

EXPERIMENTAL

Plants. The cuttings of *Tripterygium wilfordii* were imported from Taiwan in October, 1967 and 1968, and planted under glass in pots with a mixture of clay loam and vermiculite in the Horticulture Department, Oklahoma State University, Stillwater, Oklahoma. Plants were propagated by cuttings. 2-year-old plants with similar appearance, fresh weight of roots from 15–18 g and that of leaves and stems from 10–14 g, were used.

Radioactive compounds. Nicotinic acid-6-^{14}C was purchased from Nuclear Chicago Corporation and purified before use by preparative TLC in *n*-BuOH–HOAc–H_2 (4:1:1) and *n*-BuOH satd. and *n*-butanol with 3% NH_4OH. The purified nicotinic acid-6-^{14}C had a constant specific activity of 20·6 mCi/mM. Nicotinamide-carbonyl-^{14}C-adenine-dinucleotide (specific activity of 26·2 mCi/mM) was purchased from Nuclear Chicago Corporation and used without further purification.

Isolation and purification of alkaloids. A modification of Beroza's[1] method was used. Air-dried, finely grounded root powder was exhaustively extracted with CH_2Cl_2 in a Soxhlet. The solvent was removed *in vacuo* below 40° and the residue was dissolved is a min vol of ether and thoroughly mixed with an equal vol. of 5% HCl. After removal of the ether, the remaining yellowish water phase was filtered. Extraction of the tarry residue with ether and HCl was repeated until a Dragendorff test of the extract spotted on a TLC plate

[14] L. A. HADWIGER, S. E. BADIEI, G. R. WALLER and R. K. GHOLSON, *Biochem. Biophys. Res. Commun.* **13,** 466 (1963).
[15] S. TAKEI, T. TOTSU and K. NAKANISHI, *Agric. Biol. Chem.* **30,** 169 (1966).
[16] I. J. RYRIE and K. J. SCOTT, *Biochem. J.* **115,** 679 (1969).
[17] R. K. GHOLSON, *Nature, Lond.* **212,** 933 (1966).

was negative. The acid extract was cooled to about 4° and treated with conc NH_4OH to pH 9, the temp. being kept below 4°. Several hours later, the precipitated alkaloid was filtered, washed with cooled distilled water and dried. The dry crude alkaloid (yield 0·25%) was dissolved in acetone and used for chromatography.

Saponification and separation of alkaloid components. 50 mg of the alkaloid fraction isolated from TLC was hydrolyzed with 1 ml of 1 M KOH in diethylene glycol for 30 min at 125° and the saponification mixture was fractionated by liquid–liquid extraction at various pHs, as described by Beroza.[2] Wilfordic acid and hydroxywilfordic acid were converted to their methyl esters with CH_2N_2.

Low and high resolution MS. Low resolution MS were obtained on a prototype of the LKB-9000 GLC-MS apparatus.[18] MS were obtained either using the direct inlet system or gas liquid chromatography. High resolution MS analyses were conducted by K. Biemann and C. Hignite in the Mass Spectrometry Laboratory, Chemistry Department, Massachusetts Institute of Technology.

Administration of labelled compounds. Labelled compounds were administered using a micro syringe, in the upper part of the soft stems and the desired amount of labelled compound was injected slowly. Uptake of 50 μl of solution was usually completed within 15 min. Plants were divided into stems and leaves, and into roots. The divided plant part was weighed, frozen with liquid N_2, homogenized with Virtis '23' omnimizer in 80% methanol and filtered using a sintered glass funnel (medium porosity). Extraction with methanol was repeated four times until the remaining material was free of soluble pigments. The solvent was removed from the pooled extracts by evaporation at 40° under reduced pressure. The residue was dissolved in a min. vol. $CHCl_3$ or Et_2O and mixed with an equal vol. distilled water. Upon removal of the organic solvent at room temp. under reduced pressure, the remaining yellowish aqueous solution containing polar compounds was filtered. This extraction was repeated four times. The aqueous phase was reduced in volume and applied to an anion exchange column. The residue remaining in the flask and on the filter paper after water extraction was extracted with acetone and used for TLC.

Anion exchange column chromatography. A portion of the aqueous phase extract containing the polar metabolites of interest was placed on a Dowex 1-X8 formate column, 1·5 × 30 cm. Elution was by application of a formic acid concentration gradient initially with 150 ml H_2O in the mixing chamber, into which 150 ml of deionized water, 250 ml of 0·25 N formic acid, 250 ml of 2 N formic acid and 250 ml of 4 N formic acid were successively introduced. A fraction collector equipped with an ISCO UA-2 flow UV analyzer, 254 nm, was adjusted at the rate of about 35 ml/hr. The radioactivity of the pooled 10 ml fractions collected from the anion exchange column was measured using 1 ml of aqueous solution from each test tube in 10 ml Bray's scintillation solution.

Partition column chromatography. The acetone extract from the TLC plate was chromatographed on a silicic acid column. The immobile solvent, dilute HCl, was equilibrated with the mobile solvent, ether, at the column temperature (10°) by means of a steady flow of cooled tap water. The column was prepared from 25 g silicic acid, and 14 ml 0·6% HCl. The alkaloid fraction dissolved in 30 ml of ether was added and washed into the gel with several portions of ether. The flow rate of the column was adjusted to 60 ml/hr and 10 ml fractions of the effluent were collected. The absorbancy of each fraction was measured at 270 and 255 nm as described by Beroza.[1]

TLC. Plates, 0·75 nm thick, prepared from Silica Gel PF 254 + 366 (Merck Co.), were developed with acetone–hexane (4:1). The alkaloids were detected either by observing their fluorescence at 254 nm or by spraying with Dragendorff reagent. The radioactivity on the chromatograms was located with a Nuclear Chicago 4 π Actigraph-III strip counter. The alkaloid zone on the plates was scraped off, put in a small column and eluted with acetone and used for further studies.

Paper chromatography. The fractions from the Dowex 1-X8 column containing radioactive products from the anion exchange column were combined and lyophilized to dryness. The residue was dissolved in distilled water and then spotted on Whatman No. 1 paper with authentic compounds and descending chromatography was carried out in 1 M NH_4OAc–EtOH (3:7) adjusted to pH 5·0 with HCl and in isobutyric acid–NH_4OH–H_2O (66:1·7:33, pH 2·8). A Nuclear Chicago 4 π chromatogram scanner and UV lamp (254 nm) were employed to locate radioactive and quenching spots respectively.

Acknowledgements—The authors thank Dr. M. Beroza for providing *T. wilfordii* alkaloids. Appreciation is extended to Dr. C. Hignite, Dr. K. Biemann, and Mr. K. F. Kinneberg for their assistance in obtaining the mass spectra, Dr. P. F. Flanagan for obtaining the NMR spectra.

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