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AZETIDINE-2-CARBOXYLIC ACID DERIVATIVES FROM SEEDS OF *FAGUS SILVATICA* L. AND A REVISED STRUCTURE FOR NICOTIANAMINE*

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Key Word Index—*Fagus silvatica*; Fagaceae, beechnuts, *N*-(3-amino-3-carboxypropyl)azetidine-2-carboxylic acid, *N*-[*N*-(3-amino-3-carboxypropyl)-3-amino-3-carboxypropyl]azetidine-2-carboxylic acid, azetidine-2-carboxylic acid, nicotianamine, non-protein amino acids, C_4-amino acids, imino acids

Abstract—2(*S*),3′(*S*)-*N*-(3-Amino-3-carboxypropyl)azetidine-2-carboxylic acid and 2(*S*),3′(*S*),3″(*S*)-*N*-[*N*-(3-amino-3-carboxypropyl)-3-amino-3-carboxypropyl]azetidine-2-carboxylic acid have been isolated from seeds of *Fagus silvatica* L. (beechnuts) The structures have been established by PMR- and ^{13}C-NMR-spectroscopy and by synthesis from L-azetidine-2-carboxylic acid The second of the new amino acids is identical with nicotianamine, previously isolated from *Nicotiana tabacum* but assigned a different formula The ring opening reactions of azetidine-2-carboxylic acid in neutral solution have been studied and the chemical and possibly biochemical precursor role of this amino acid for various amino acids including the two new ones described here, nicotianine [*N*-(3-amino-3-carboxypropyl)nicotinic acid] and methionine is discussed

INTRODUCTION

IN THE course of a study of the free amino acids in seeds of *Fagus silvatica* L. two new amino acids were obtained.[1,2] The present paper describes the identification of these two amino acids as 2(*S*),3′(*S*)-*N*-(3-amino-3-carboxypropyl)azetidine-2-carboxylic acid (**1**) and 2(*S*),3′(*S*),3″(*S*)-*N*-[*N*-(3-amino-3-carboxypropyl)-3-amino-3-carboxypropyl]azetidine-2-carboxylic acid (**2**) **2** Is identical with nicotianamine which has previously been isolated from *Nicotiana tabacum* and for which the isomeric structure **3** has been proposed.[3] Both **1** and **2** can be produced chemically from azetidine-2-carboxylic acid (**4**) by ring opening. The possibility of ring opening of **4** has therefore been studied, and the chemical and possibly biochemical precursor role of **4** for various amino acids including **1**, **2**, nicotianine [*N*-(3-amino-3-carboxypropyl]nicotinic acid, **5**),[4] and methionine is discussed.

METHODS AND RESULTS

The partial isolation of **1** and **2** has been described in the previous communication.[2] Final purification was accomplished by preparative paper chromatography and crystallization from water to produce chromatographically and analytically pure samples both having the elemental composition ($C_4H_7NO_2$)

* Taken in part from the thesis of I Kristensen, Copenhagen (1973)[1]

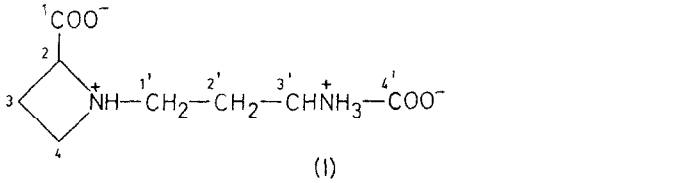
[1] KRISTENSEN, I (1973) Free Amino Acids and γ-Glutamyl Peptides in *Fagus silvatica* L, Thesis, The Royal Veterinary and Agricultural University, Copenhagen

[2] KRISTENSEN, I, LARSEN, P O and SØRENSEN, H (1974) *Phytochemistry* **13**, 2803

[3] NOMA, M, NOGUCHI, M and TAMAKI, E (1971) *Tetrahedron Letters*, 2017

[4] NOMA, M, SAKUMA, H and TAMAKI, E (1968) *Phytochemistry* **7**, 1861

The results obtained by ^{13}C-NMR-spectroscopy for **1**, **2**, **4** in strong base, and *N*-methylazetidine (**6**) are displayed in Table 1. The two carboxyl groups in **1** appear as only one peak. Otherwise the numbers of C-atoms observed are in agreement with the structures proposed. Also the chemical shifts and the number of hydrogen atoms on each C-atom are in agreement with the proposed structures.



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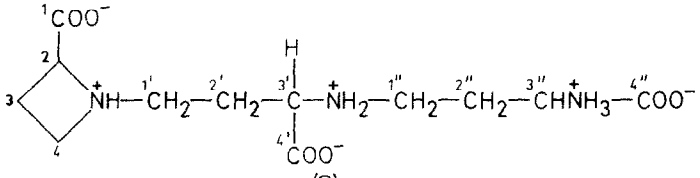

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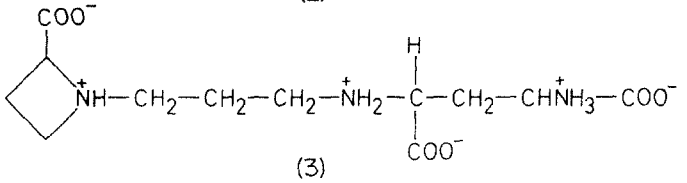

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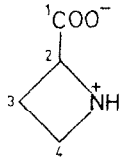
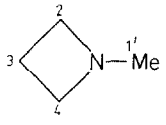


(4)



(6)

The results obtained by PMR-spectroscopy for **1** and **2** are displayed in Table 2. The spectrum of **2** is identical in all respects with that published for nicotianamine.[3] However the coupling patterns established by decoupling experiments are clearly incompatible with structure **3**. The signals for the γ-protons in the azetidine part of the molecules are hidden in the DOH-bands. The same is observed for **4**.[3, 5] The spectrum of **1** contains two CH_2–CH_2–CH spin systems, that of **2** three. The spin systems assigned to the ring part of the molecules are similar to the known system of **4**.[3, 6] The IR spectrum of **2** is identical in all respects with that of an authentic sample of nicotianamine.[3]

Both **1** and **2** reacted as α-amino acids when treated with ninhydrin after masking with cupric ions.[7] Paper electrophoresis at various pH-values indicated that pK_3 for **1** and pK_4

[5] MACIEL, G. E. and SAVITSKY, G. B. (1965) *J. Phys. Chem.* **69**, 3925.
[6] THOMAS, W. A. and WILLIAMS, M. K. (1972) *Org. Magn. Resonance* **4**, 145.
[7] LARSEN, P. O. and KJAER, A. (1960) *Biochim. Biophys. Acta* **38**, 148.

TABLE 1 ^{13}C-NMR-SPECTROSCOPIC DATA FOR *N*-(3-AMINO-3-CARBOXYPROPYL)AZETIDINE-2-CARBOXYLIC ACID (**1**), *N*-[*N*-(3-AMINO-3-CARBOXYPROPYL)-3-AMINO-3-CARBOXYPROPYL]AZETIDINE-2-CARBOXYLIC ACID (**2**), AZETIDINE-2-CARBOXYLIC ACID (**4**), AND *N*-METHYLAZETIDINE (**6**)

	1		**2**		**4**		**6***
Carbon atom	δ	Spin–spin splitting	δ	Spin–spin splitting	δ in D_2O	δ with excess NaOH	δ
1	[174·9]	*s*	[174·9]	*s*	174·9	183·5	—
4′	174·9	*s*	174·7	*s*	—	—	—
4″	—	—	173·6	*s*	—	—	—
2	69·0	*d*	69·0	*d*	59·9	60·3	57·9
3′	54·5	*d*	61·6	*d*	—	—	—
3″	—	—	54·8	*d*	—	—	—
3	23·4	*t*	23·3	*t*	24·2	26·7	17·7
2′	27·9	*t*	29·3	*t*	—	—	—
2″	—	—	27·0	*t*	—	—	—
4	53·6	*t*	53·2	*t*	43·7	43·4	57·9
1′	52·7	*t*	52·7	*t*	—	—	(46·6)
1″	—	—	46·2	*t*	—	—	—

The spectra were recorded in D_2O at 22·63 MHz on a Bruker HX 90E instrument using the pulse technique with Fourier transformation. Spin–spin splittings indicating the number of H-atoms on each C-atom were obtained for **1** and **2** from partially decoupled spectra. *s* Singlet, *d* doublet, *t* triplet. δ-Values are in ppm downfield from TMS. Dioxane was used as internal standard in the measurement of the spectra of **4** [δ(TMS) = δ(dioxane) + 67·4 ppm]. No internal standard was used in the measurement of the spectra of **1** and **2**, but the δ-value for the COO^--group at lowest field was assumed to be at 174·9 ppm. For designations of atoms see Formula chart. For azetidine itself (Eastman-Kodak) was found δ 22·4 and 48·2 (neat liquid), 21·8 and 47·3 (10% sol. in D_2O, and 19·3 and 47·7 (10% sol. of the hydrochloride in D_2O).

* Ref. 5, measurement on neat liquid.

for **2** are about 7. The high value of the macroscopic dissociation constant can partly be explained by the proximity of the positive charges [the pK_2- and pK_3-values for 2,4-diaminobutyric acid are 8·28 and 10·50 (Ref. 8)], partly by the alkylation of the azetidine nitrogen [azetidine itself has a pK_a value of 11·29, whereas *N*-methylazetidine has a pK_a

TABLE 2 PMR-SPECTROSCOPIC DATA FOR *N*-(3-AMINO-3-CARBOXYPROPYL)AZETIDINE-2-CARBOXYLIC ACID (**1**) AND *N*-[*N*-(3-AMINO-3-CARBOXYPROPYL)-3-AMINO-3-CARBOXYPROPYL]AZETIDINE-2-CARBOXYLIC ACID (**2**)

	1		**2**	
Hydrogen atom	Signal (δ ppm)	Effect of irradiation	Signal (δ ppm)	Effect of irradiation
2	4·7	Changes at 2·4–2·9	4·7	Changes at 2·4–2·9
3a + 3b	2·4–2·9	Changes at 3·7–4·1	2·4–2·9	Changes at 3·7–4·1
4a + 4b	3·7–4·2		3·7–4·2	Triplet at 2·4–2·9
1′a + 1′b	3·42 (*t*, *J* 7·5 Hz) 3·46 (*t*, *J* 7·5 Hz)	Doublet at 2·17	3·42 (*m*)	Changes at 2·0–2·4
2′a + 2′b	2·14 (two triplets, *J* 7 and 5·5 Hz)	Broad singlet at 3·45, singlet at 3·91	2·0–2·4 (*m*)	Singlets at 3·29, 3·42, 3·83 and 3·90
3′	3·91	Triplet at 2·14	3·83	
1″a + 1″b	—	—	3·29 (*t*)	Changes at 2·0–2·4
2″a + 2″b	—	—	2·0–2·4 (*m*)	Singlets at 3·29, 3·42, 3·83 and 3·90
3″	—	—	3·90 (*t*)	

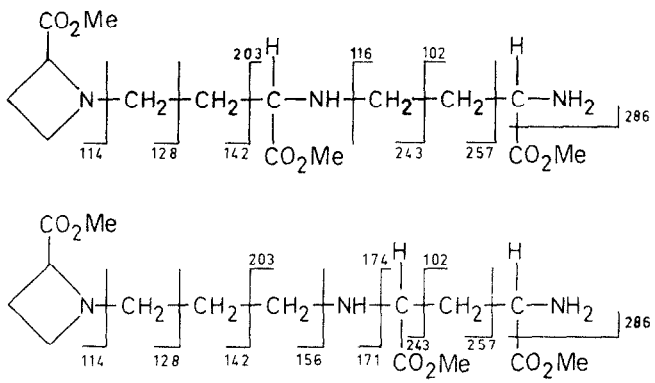
The spectra were recorded in D_2O at 90 MHz on a Bruker HX 90E instrument using the pulse technique and Fourier transformation. δ-Values are in ppm downfield from sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate. For designation of atoms see Formula chart.

[8] ALBERT, A. (1952) *Biochem. J.* **50**, 690

value of 10·40 (Ref. 9)] and partly by the statistical effect due to the presence of two or three ammonium groups in the molecules. The pK_2 value for **4** has never been determined with accuracy but paper electrophoresis at various pH-values indicates that it is about 10.

The structures **1** and **2** were further corroborated by synthesis from **4** and the configuration at all centers established as L (or *S*) (see below)

Structure **3** for nicotianamine was originally proposed partly on account of the mass spectrum of the trimethyl ester of this compound.[3] In Scheme 1 are shown the trimethyl esters corresponding to structures **2** and **3** with the expected cleavages indicated Mass spectroscopic distinction between the two structures is not easy However the strong peaks in the spectrum published[3] at *m/e* 142 and 204 favour structure **2** The peak at *m/e* 204 may originate from *β*-cleavage with *γ*-hydrogen rearrangement in structure **2** In conclusion the combined evidence including that previously reported for nicotianamine[3] is in agreement with structure **2**, whereas especially the NMR-data and the synthesis from **4** are incompatible with structure **3**



SCHEME 1 STRUCTURES OF THE TRIMETHYL ESTER OF *N*-[*N*-(3-AMINO-3-CARBOXYPROPYL)-3-AMINO-3-CARBOXYPROPYL]AZETIDINE-2-CARBOXYLIC ACID (**2**) AND OF THE TRIMETHYL ESTER CORRESPONDING TO THE PREVIOUSLY PROPOSED STRUCTURE (**3**) FOR NICOTIANAMINE
Expected mass spectrometric cleavages are indicated

Obviously **1** and **2** are polymerization products of **4** **4** is known to be stable in strong base[10] just like azetidine itself and other azetidine derivatives.[11,12] Ring opening by nucleophilic attack is therefore not possible when the ring nitrogen atom is uncharged but only when it is in the protonated, positively charged form giving electrophilic assistance to the reaction In hydrochloric acid, **4** is decomposed to give homoserine (**7**), 4-amino-2-hydroxybutyric acid (**8**), 2-amino-4-chlorobutyric acid (**9**), and 4-amino-2-chlorobutyric acid (**10**) [10] The acid-catalyzed ring opening of azetidine itself and of other azetidine derivatives is well-known [11,12] The stability of **4** at neutral pH-values has not previously been studied closely

When an aqueous solution of **4** was heated to 100° for 24 hr, PC analysis of the reaction mixture revealed the presence of **1**, **2**, and **7** whereas no **4** remained The production of

[9] SLARLES, S., TAMRLS, M., BLOCK, F and QUARTERMAN L. A. (1956) *J. Am. Chem. Soc* **78**, 4917
[10] FOWDEN, L. (1956) *Biochem J* **64**, 323
[11] MOORE, J. A. (1964) in *Heterocyclic Compounds with Three- and Four-Membered Rings*. PART TWO (WEISSBERGER, A., ed.), p. 885 Interscience, New York
[12] GAIRTNER, V. R. (1969) *J. Heterocycl. Chem* **6**, 273

1 and **7** must take place by nucleophilic attack on a molecule of **4** with positively charged nitrogen by a molecule of **4** with uncharged nitrogen or H_2O or OH^- ions. **2** must be produced by attack on the ring in **1** by **4** or less likely by attack on **4** by the primary amino group of **1**. Isomers of **1** and **2** could conceivably be formed by 1,2-fission of the ring. These isomers would however contain primary amino groups not α to the carboxyl groups. No such compounds could be identified by use of a specific paper-chromatographic test for non-α-amino acids.[7] Apparently for steric reasons the isomers of **1** and **2** are formed in very small amounts or not at all. The production of small amounts of **8** was not excluded.

Since in water the amount of **4** with uncharged nitrogen must be very small heating was attempted of a solution of **4** containing half an equivalent of sodium hydroxide. As expected this resulted in increased yields of **1** and **2** and decreased yields of **7**. This procedure was used for a microsynthesis of **1** and **2** Heating of an aq soln of 100 mg of L-**4** with half an equivalent of NaOH to 100° for 24 hr followed by preparative PC and ion exchange purification resulted in the isolation of **1**. The identity with the natural product was established by determination of optical rotation and infrared spectrum. Also **2** was isolated although in rather small amounts The identity with the natural product was established by determination of optical rotation and PMR-spectrum, whereas the infrared spectrum of the semicrystalline material was of low quality The synthesis from L-**4** and the identity of optical rotations established the configurations at all centers as L (or *S*) During the preparative paper chromatography of the reaction mixture additional compounds with smaller R_f values than **1** and **2** were observed These compounds presumably are similar polymerization products containing four or more C_4-units.

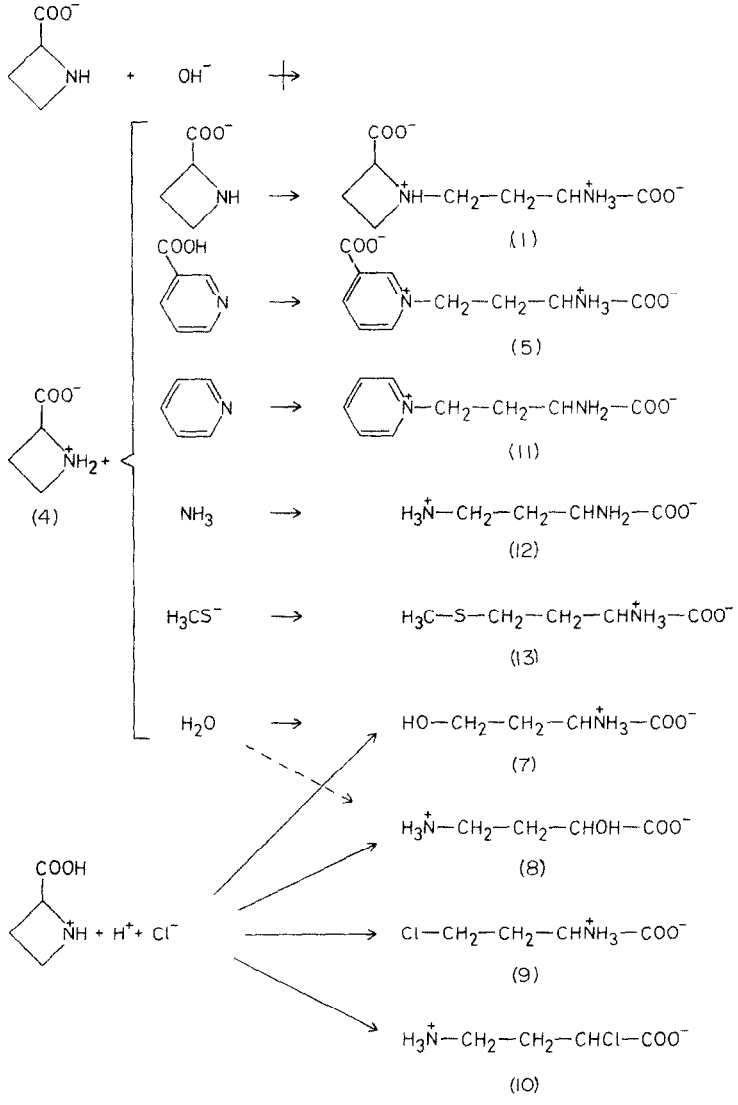
The polymerization of hydrolysis of **4** in H_2O is not fast; 24 hr at 100° resulted in complete conversion of **4**. Reflux of a solution of **4** in 70% aq. methanol for 24 hr did not result in the production of **1**, **2**, or **7** in detectable amounts. A reference solution of **4** in H_2O kept in the refrigerator for 5 years was still chromatographically pure.

Other nucleophiles may be used for ring opening of **4**. Heating in 1 N aq. pyridine resulted in the production of a new amino acid identified only by PC but assumed to be *N*-(3-amino-3-carboxypropyl)pyridinium betaine (**11**). Heating with nicotinic acid resulted in the production of **5**, a compound previously isolated from *Nicotiana tabacum* and named nicotianine [4] The identity was proved by isolation of the reaction product and comparison of optical rotation and IR spectrum with those published for nicotianine.[4] Heating of **4** in 1 N NH_3 resulted in the production of 2,4-diaminobutyric acid (**12**), identified by paper chromatography.

Use of amino groups from other amino acids as nucleophilic reagents was tried. Heating of **4** in H_2O with either alanine, γ-aminobutyric acid, or asparagine resulted however mainly in the production of **7**. Heating of the same solutions but with the addition of half an equivalent of NaOH resulted in increased yields of **1** and **2**. Apparently the amino groups in alanine, γ-aminobutyric acid, or asparagine are not competitive with the amino group from **4** itself.

Heating of an aqueous solution of **4** and methyl mercaptan with half an equivalent of NaOH resulted in nearly complete conversion into methionine (**13**) as revealed by paper chromatography. The methyl mercaptide ion is therefore a better nucleophile for the ring opening than **4** itself.

Heating of an aqueous solution of **4** with equimolar amounts of potassium cyanide only produced **1**, **2**, and **7**. The cyanide ion is therefore not able to compete with **4** as a nucleophile. The various reaction possibilities for **4** are outlined in Scheme 2.



SCHEME 2 REACTION POSSIBILITIES FOR AZETIDINE-2-CARBOXYLIC ACID
The reaction with water to give **7** and **8** and the dimerization to give **1** always compete with the other possibilities

Since **1** and **2** are produced easily from **4** by chemical reaction the possibility has been considered that they are artefacts produced during the isolation procedure from the beechnuts. This can however be excluded for several reasons The large-scale isolation which gave pure **1** and **2** involved reflux with 70% methanol for some hours, a number of evaporations under reduced pressure and the use of aq. ammonia or pyridine on ion exchange resins.[2] **4** is however stable for 24 hr in refluxing 70% methanol Furthermore a partial isolation was performed without the use of heat in any steps.[2] **2** could already be identified on paper chromatograms in the total amino acid fraction obtained by elution of the first

ion exchange resin with pyridine. Both **1** and **2** could be identified by PC in the fraction of neutral amino acids obtained after passage of the total amino acid fraction through a basic resin in the acetate form and subsequent fractionation on an acid resin in the 3-chloropyridinium form by elution with aq. 3-chloropyridine **4** and its main decomposition product in H_2O, **7**, were not identified in any of the fractions from either the large-scale or the small-scale isolation. It seems impossible that transformation of **4** into **1** and **2** could take place under the experimental conditions without leaving any traces of **4** behind and without the concomitant production of **7**, especially when it is considered that **4** has been isolated from plant material several times, sometimes under more vigorous conditions than those used in the present study.[13] We are therefore led to believe that **1** and **2** are true constituents of *F. silvatica*.

DISCUSSION

4 has been found in various species of Liliaceae and Agavaceae,[10] Chenopodiaceae[13] and Leguminosae.[14] The occurrence of the two derivatives of **4** in *F. silvatica* is therefore not too surprising. **1** and **2**, even though they are true natural constituents, probably derive from **4**.

The structure **2** for nicotianamine also shows that this compound from *Nicotiana tabacum* is a derivative of **4**; the same is therefore rendered likely for **5**. So few details are however given about the procedures used in the isolation of nicotianamine and **5** that it cannot be totally excluded that they are artefacts produced from **4** and from **4** and nicotinic acid It would therefore be interesting to know if **4** itself could be identified in *N. tabacum*.

The biosynthesis of **4** has been studied several times. It is natural to assume that a properly activated C_4-precursor is involved. *S*-Adenosylmethionine has been proposed as a possible precursor.[15,16] More recent investigations indicate a pathway via 2,4-diaminobutyric acid and 4-amino-2-oxobutyric acid.[17,18] The present work, however, emphasizes that **4** is itself an activated C_4-compound which can act as precursor for a number of amino acids, provided proper nucleophilic attack takes place. **1**, **2**, and **5** are among natural amino acids that can derive from **4**, but other possible candidates are homoserine, 2,4-diaminobutyric acid, methionine, and homocysteine. Attack by a nucleophilic C-atom can also not be totally excluded No information is available on the transformation of **4** in plants, even though there are no reasons to believe that it is an end product. In experiments with leaves from *Convallaria majalis* it was however found that **4** was only slowly degraded.[19]

The reaction type producing **1** and **2** from **4** is a new principle for the establishment of imino bonds. Such bonds are present in various amino acids but in most cases they seem to be formed by reduction of Schiff bases produced from amino acids and keto acids. Imino bonds are also present in polyamines like for example spermidine and spermine. These compounds are biosynthetically derived from putrescine and *S*-adenosyl-3-methylmercaptopropylamine.[20]

[13] FOWDEN, L (1972) *Phytochemistry* **11**, 2271
[14] SUNG, M -L and FOWDEN, L (1969) *Phytochemistry* **8**, 2095
[15] SCHLENK, F and DAINKO, J L (1960) U S Atomic Energy Commission Reports of the Argonne National Lab ANL-6200, 94
[16] LEETE, E J (1964) *J Am Chem Soc* **86**, 3162
[17] SUNG, M -L and FOWDEN, L (1971) *Phytochemistry* **10**, 1523
[18] LEETE, E , DAVIS, G E , HUTCHINSON, C R , WOO, K W and CHEDEKEL, M R (1974) *Phytochemistry* **13**, 427
[19] FOWDEN, L and BRYANT, M (1959) *Biochem J* **71**, 210.
[20] TABOR, H and TABOR, C W (1972) *Adv Enzymol* **36**, 203

EXPERIMENTAL

General methods and instrumentation have been described in the previous communication.[2] Microanalyses were performed by Mr. G. Cornah and his staff whereas the ultramicroanalysis was performed by Mr. G. Hesselius, Uppsala, Sweden. **1** Was isolated from fraction (L 1–4) described in the previous communication by preparative PC in solvent 1 (Ref. 2) (yield 10 mg). Purification on a small ion exchange column and crystallization from EtOH–H_2O (9:1) produced an analytical sample (7 mg). [Found: C, 47.25; H, 7.00; N, 13.55. $(C_4H_7NO_2)_n$ requires: C, 47.54; H, 6.93; N, 13.86%. (ultramicroanalysis)]. IR: ν_{max}^{KBr} 3400 cm^{-1} (medium), 3050 (*m*), 2930 (*m*), 2850 (*m*), 2130 (weak), 1590–1650 (strong), 1530 (*m*), 1450 (*w*), 1410 (*s*), 1365 (*m*), 1330 (*s*), 1255 (*w*), 1230 (*w*), 1185 (*w*), 1130 (*w*), 1095 (*w*), 1065 (*w*), 1030 (*w*), 1000 (*w*), 970 (*w*), 955 (*w*), 910 (*w*), 870 (*w*), 845 (*w*), 815 (*w*), 795 (*w*), 760 (*m*), 705 (*w*), 655 (*w*), 565 (*m*), 450 (*w*), 425 (*w*), 345 (*w*), 300 (*m*). $[\alpha]_D^{20}$ −83° (*c* 0.8, H_2O). R_f in solvent 1 (Ref. 2) 0.09, in solvent 2, 0.76. M.p. decomp. above 230°. For ^{13}C-NMR- and PMR-data see Tables 1 and 2. **2** Was isolated from fraction (L 1–6) described in the previous communication by preparative paper chromatography in solvent 1 (Ref. 2) (yield 35 mg). Purification on a small ion exchange column and crystallization from water produced an analytical sample (13 mg). (Found: C, 42.49; H, 7.28; N, 12.42. H_2O, 11.1. $C_{12}H_{21}N_3O_{10}$, $2H_2O$ requires: C, 42.47; H, 7.43; N, 12.38; H_2O, 11.9%. The H_2O content was determined by drying an air-dried sample over P_2O_5 at 50°. The H_2O was regained on exposing the sample to the atmosphere. The H_2O of crystallization has not been noted previously.) IR: ν_{max}^{KBr} 3420 cm^{-1} (*s*), 3040 (*s*), 2870 (*m*), 2600 (*m*), 1615 (*m*), 1600 (*s*), 1575 (*s*), 1500 (*w*), 1470 (*m*), 1415 (*s*), 1375 (*s*), 1350 (*m*), 1295 (*s*), 1240 (*w*), 1215 (*m*), 1190 (*w*), 1130 (*m*), 1110 (*m*), 1080 (*m*), 1055 (*m*), 1000 (*w*), 965 (*w*), 940 (*w*), 880 (*w*), 835 (*w*), 800 (*s*), 765 (*m*), 670 (*m*), 570 (*s*), 490 (*m*), 435 (*w*), 420 (*s*), 365 (*s*). The spectrum was identical with that of an authentic sample.[4] $[\alpha]_D^{22}$ −50° (*c* 0.4, H_2O). Lit. value for nicotianamine: $[\alpha]_D^{23}$ −60.5° (*c* 2.7, H_2O).[3] R_f in solvent 1 (Ref. 2) 0.02, in solvent 2, 0.67. M.p. decomp. above 238°. For ^{13}C-NMR- and PMR-data see Tables 1 and 2.

Syntheses of **1** *and* **2** *from* L-**4**. 101 mg of L-**4** (1 mmol) in 5 ml of 0.1 N NaOH was refluxed for 24 hr. The reaction mixture was subjected to preparative PC in solvent 1 (Ref. 1) on Whatman No. 1 paper for 3 days. The bands corresponding to **1** and **2** were eluted with water (yields 25 and 6 mg).

Further purification of **1** was accomplished by use of a small ion exchange column (25 mg) and crystallization from EtOH–H_2O (16 mg). $[\alpha]_D^{20}$ −79° (*c* 1, H_2O). IR identical with that found for the natural material.

2 Was likewise purified by passage through a small ion exchange column (6 mg) and crystallization from EtOH–H_2O (4 mg). $[\alpha]_D^{20}$ −45° (*c* 0.2, H_2O). The PMR-spectrum was identical with that found for the natural material.

Synthesis of nicotianamine (**5**) from L-**4**. 101 mg of L-**4** (1 mmol) and 124 mg of nicotinic acid (1 mmol) were refluxed in 5 ml of H_2O for 3 days. The reaction mixture was subjected to preparative PP in solvent 1 (Ref. 2) for 3 days. The band corresponding to **5** was eluted with H_2O (54 mg). Further purification was accomplished by use of a small ion exchange column (47 mg) and crystallization from MeOH–H_2O (35 mg). $[\alpha]_D^{20}$ +23.3° (*c* 1.1, H_2O). Lit. value: $[\alpha]_D^{24}$ +24.0° (*c* 2.0, H_2O).[4] IR identical with that published for nicotianamine.[4]

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