Tetrahedron Letters Vol. 21, pp 141 - 144
0040-4039/80/0108-0141$02.00/0

INTERCONVERSION OF THE ENAMINE AND IMMONIUM FORM OF CATHENAMINE

Peter Heinstein*, Joachim Stoeckigt and Meinhart H. Zenk

Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität Bochum, 463 Bochum, W. Germany

Abstract: From the amount of deuterium incorporated during the reduction of cathenamine to tetrahydroalstonine, the enamine and immonium ion form of cathenamine was demonstrated. The two forms could be interconverted depending on the presence or absence of SO_4^{-2}.

Cathenamine (3a) has been identified as a key intermediate[1] in the biosynthetic formation of monoterpene indole alkaloids like ajmalicine (4), 19-epi-ajmalicine (5) and tetrahydroalstonine (6) from tryptamine (1) and secologanin (2).

For the biosynthetic reduction of (3a) to the heteroyohimbine derivatives (4-6) an equilibrium between the enamine (3a) and the immonium ion (3b) form has been postulated[1].

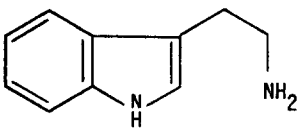
In a TLC solvent system such as petroleum ether (30-60°)-acetone-diethylamine (70:20:10) (on Sil G/UV_{254}, Macherey-Nagel, Co.,) (3a) had a RF of 0.5. However when [^{14}C]-(3a) was isolated and rechromatographed in the same TLC system considerable radioactivity, up to 25% of the applied [^{14}C]-(3a) migrated with an RF considerably higher. Similarly when enzyme incubation mixtures of *Rauwolfia verticillata* cell cultures using [1-^{14}C] (1) and (2) as substrates were chromatographed in the TLC system above, besides other radioactive products, two radioactive substances which behaved like [^{14}C]-cathenamine[2] migrated with an RF of 0.5 and 0.8, respectively. In the latter case the distribution of radioactivity varied between 70-90% slower migrating and 10-30% faster migrating material. Mass spectral analysis of the two compounds gave identical fragmentation pattern and agreed with the spectra reported previously for (3a)[1,3,4]. Since an equilibrium between the enamine form (3a) and the immonium ion form (3b) of cathenamine could have been a possible explanation for the apparent interconversion described above, attempts were made to test this interconversion through deuterium incorporation as shown in the scheme.

Radioactive (3a) was prepared and purified as described previously[2]. Treatment with a divalent anion as for instance SO_4^{-2} caused an increased formation of the faster migrating form. Typical values were 58%-69% of RF 0.8 compound depending on the cation associated with SO_4^{-2}.

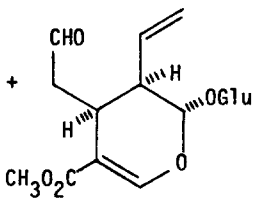
(Table I). Incubation of (3a) with H_2O, Cl^- or PO_4^{-3} buffer gave the usual ratio of slower to faster migrating compound of about 3:1.

*Present address: Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana 47907, U.S.A.

SCHEME

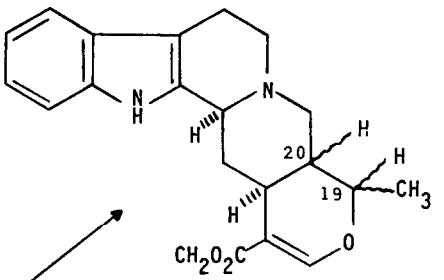


(1)

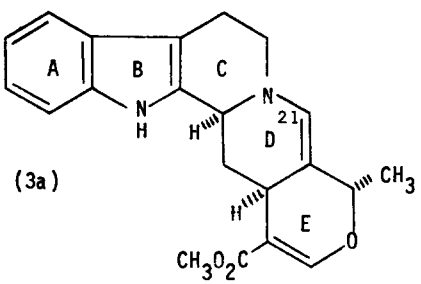


(2)

NADPH
+ H^+

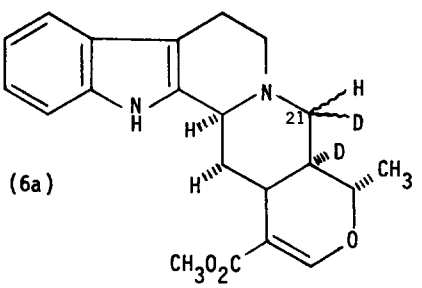


	19-H	20-H
Ajmalicine (4)	β	β
19-epi-Ajmalicine (5)	α	β
Tetrahydroalstonine (6)	β	α



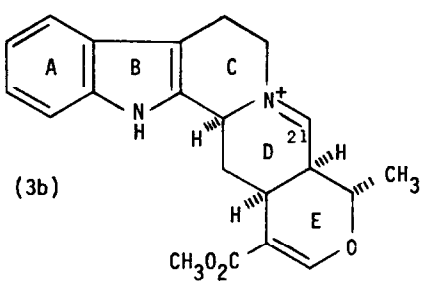
(3a)

$NaBD_4$
D_2O



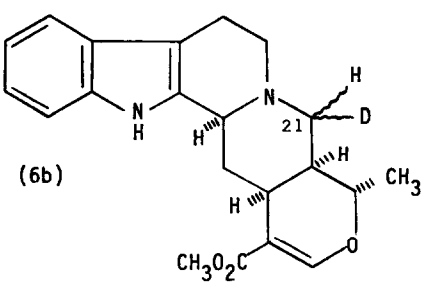
(6a)

base ⇄ SO_4^{-2}



(3b)

$NaBD_4$
D_2O



(6b)

Table I. The Formation of (3b) from Cathenamine (3a) in the Presence of Different Ions

Addition	(3a)	(3b)
	%	
Na_2HPO_4	75.7	24.3
NaCl	73.7	27.3
Na_2SO_4	41.5	58.5
$MnSO_4$	38.6	61.4
$MgSO_4$	35.6	64.4
$FeSO_4$	32.5	66.7
$CuSO_4$	31.2	68.8

[^{14}C]-cathenamine (20 nanomol, 18000 cpm) in 0.02 ml methanol and 0.05 ml of 1M K_2HPO_4-KH_2PO_4, pH 7.0, was incubated for 30 min. at 30°C with or without 5 micromol of the salts indicated, total volume 0.17 ml.

To identify the two forms of cathenamine the following incubations were carried out. In incubation A, (3a) (0.34 micromol in 0.02 ml acetonitrile) was incubated in 0.04 ml of 1M K_2HPO_4-KH_2PO_4 in D_2O (pH 7.0) and 0.1 ml D_2O. After 2 hrs at 30°C an excess of $NaBD_4$ was added. Incubation B contained 0.34 micromol of (3a) in 0.02 ml acetonitrile, 0.05 ml 1M K_2HPO_4-KH_2PO_4 (H_2O), pH 7.0, and 5 micromol of (0.1 ml) $CuSO_4$. After 2 hrs at 30° the reaction mixture was applied to a TLC plate and developed in $CHCl_3$-MeOH (100:2) with (3a) as standard. The fluorescent zone migrating slightly faster than (3a) was eluted, concentrated, taken up in 0.02 ml of acetonitrile and incubated with 0.05 ml of 1M K_2HPO_4-KH_2PO_4 in D_2O (pH 7.0) and 0.1 ml D_2O. After 10 min at 30° an excess of $NaBD_4$ was added. Both incubations were extracted with 4 x 2 volumes of ethylacetate and the product of the reduction, tetrahydroalstonine (6a and 6b)[3,4] purified through TLC (ethylacetate-ether-hexane, 20:20:8) and analyzed for deuterium by mass spectrometry (Varian MAT 111, 80 EV). The deuterium content of the two samples was calculated[5] from the molecular ions. Table II clearly showed that tetrahydroalstonine (6a) obtained from incubation A contained mainly molecules with 2 deuterium atoms, as expected when (3a) is reduced to tetrahydroalstonine (6a) (confirmed by earlier observations[6]). However, when (3a) was first treated with SO_4^{-2} in H_2O to yield (3b) and then reduced with $NaBD_4$ in D_2O to tetrahydroalstonine (6b) a large excess of molecules contained only one deuterium atom (Table II).

Table II. The Amount of Deuterium in Tetrahydroalstonine (6a and 6b) upon Reduction of Cathenamine Forms (3a and 3b) with $NaBD_4$ in D_2O Before and After Treatment with SO_4^{-2}.

Incubation*	Mole % Deuterium		
	Unlabeled	1-D	2-D
A (6a)	8.5	15.8	75.8
B (6b)	35.4	50.3	14.3

*For experimental details see text.

From the characteristic fragment at m/e 184[7] of the unlabeled species and the abundance of the fragment at m/e 185 in both the singly and the doubly labeled species it was clear that the deuterium in the singly labeled species was incorporated at position 21 of (3b).

The results are in agreement with the postulation[1] that cathenamine can exist as an enamine (3a) or as an immonium ion (3b), and that the two forms are interconvertible.

It can be argued that the biosynthetic reductive production of (4) and (6) proceeds out of the equilibrium of (3a) and (3b), while opening of ring E of (3a,b) and subsequent stereochemical rearrangement of C-19 prior to ring closure and reduction could lead to (5).

Acknowledgements

The authors thank Dr. M. Rueffer for preliminary experiments and the Bundesminister für Forschung und Technologie, Bonn, for financial support.

References

(1) J. Stoeckigt, H.P. Husson, C. Kan-Fan and M.H. Zenk, J.C.S. Chem. Somm., 1977, 164.
(2) P. Heinstein, G. Hoefle and J. Stoeckigt, Planta medica, in press (1979).
(3) H.P. Husson, C. Kan-Fan, T. Sevenet and J.P. Vidal, Tetrahedron Letters, 1977, 1889.
(4) J. Stoeckigt, Phytochemistry, 18, 965 (1979).
(5) K. Biemann, Mass Spectrometry, Organic Chemical Applications, McGraw-Hill, New York, N.Y., pp. 224-227, 1962.
(6) J. Stoeckigt, G. Hoefle and P. Heinstein, manuscript in preparation.
(7) M. Hesse, in "Indolealkaloids - Fortschritte der Massenspectrometrie," Vol. 1, (H. Budzikiewicz, Ed.), Verlag Chemie, p. 130, 1974.

(Received in USA 24 August 1979)