Tetrahedron Letters, Vol.27,No.2, pp 175-178, 1986 0040-4039/86 $3.00 + .00
Printed in Great Britain

RATIONAL SYNTHESIS OF DEUTERIUM-LABELLED PYRIDOXAL AND PYRIDOXYL ALKALOIDS[1,2]

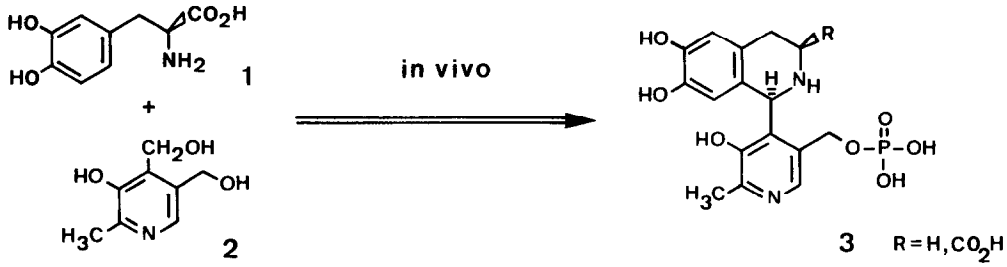
Gerhard Bringmann* and Stephan Schneider

Organisch-Chemisches Institut der Universität
Orléansring 23, D-4400 Münster, F.R. Germany

Summary: A synthesis of pyridoxal is described, which avoids delicate redox reactions at the accomplished pyridoxyl system. This synthesis allows the facile preparation of highly (>98%) deuterated pyridoxal **10b** and of B_6-derived alkaloids.

The very mild reaction conditions under which the key step of isoquinoline biosynthesis - the Mannich condensation of aryl ethyl amines with reactive carbonyl compounds - can be imitated *in vitro*[3], led to the detection that this Pictet-Spengler type reaction spontaneously also may occur in man, e.g. after alcohol consumption[4]. In particular, the aldehyde pyridoxal phosphate, the central coenzyme of the amino acid metabolism, might react non-enzymatically with endogeneous bi-nucleophiles to alkaloid-type metabolites.

We could recently demonstrate the *in vivo* formation of pyridoxyl isoquinoline alkaloids **3** in rats from L-dopa (**1**) and vitamine B_6 (**2**)[5], which are administered in unphysiologically high doses to patients suffering from metabolic diseases like parkinsonism and homocystinuria[6,7].



While these animal experiments could be achieved with radioactive isotopes, the quantitative determination of endogeneous alkaloids in man requires stable isotope labelled reference substances, which can be added to body fluids or tissue samples as internal MS standards[8]. We now wish to describe a synthesis of [CD_3]pyridoxal (**10b**) and its rational chemical incorporation into deuterated pyridoxyl alkaloids.

The known hydrogen exchange procedure on the N-benzyl salt of the prefabricated alcohol pyridoxine (**2**), followed by debenzylation[9] and oxidation, gives deuterated pyridoxal in a poor overall yield (3%). Thus, a total synthesis of deuterated pyridoxal **10b** by Diels-Alder reaction appeared to us as the more efficient alternative. In order to avoid ticklish redox reactions on the sensitive pyridoxyl system, as necessary in described syntheses of vitamine B_6[10], we used the dienophile of the central cycloaddition step immediately in the final oxidation level required - as an unsymmetrical olefin like **7**, with an alcohol and an aldehyde function.

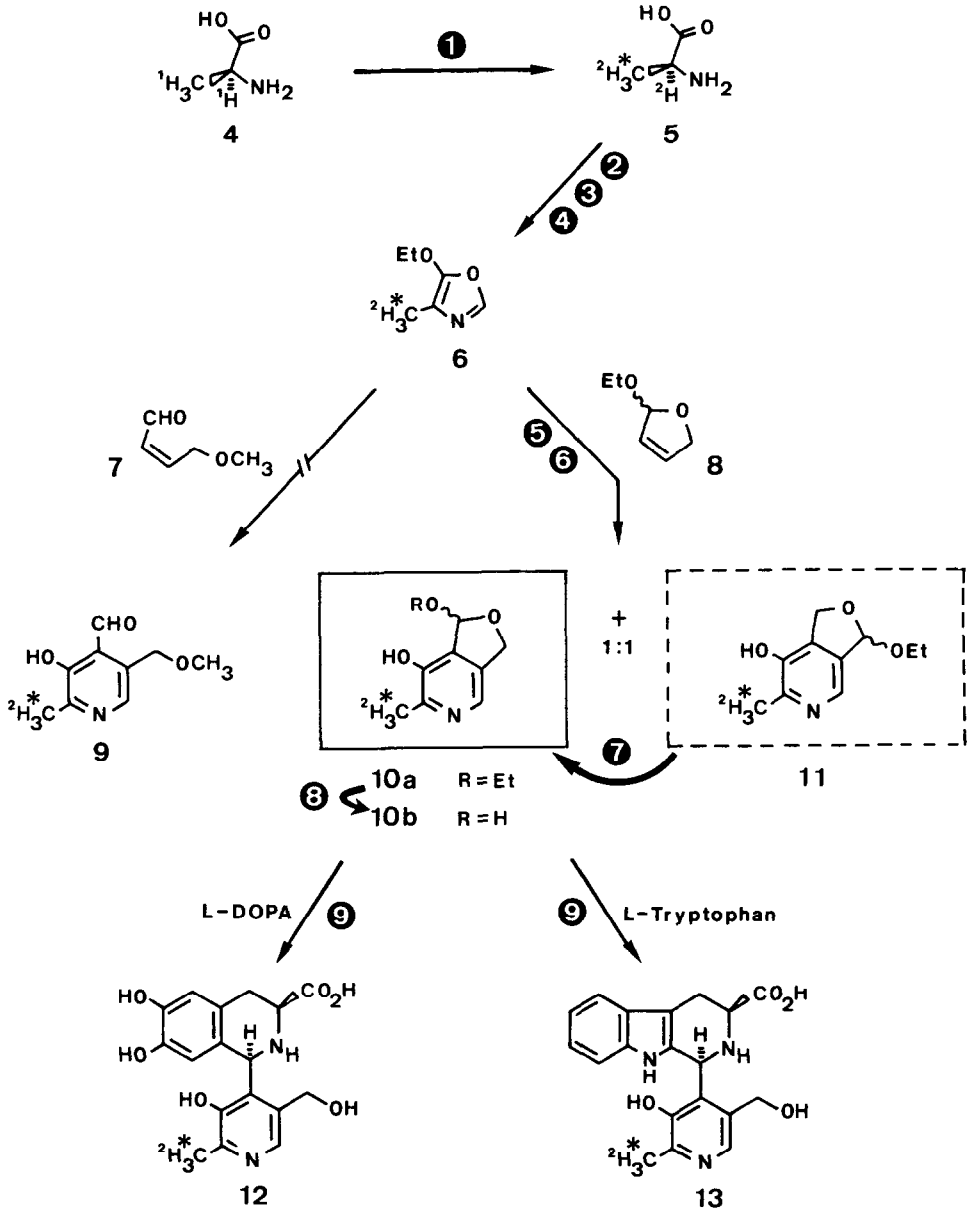
7 is easily prepared from Z-2-butene-1,4-diol, whereas the diene, the oxazole **6**, synthetically originates from alanine (**1**). This amino acid can very effectively be deuterated under the influence of a transaminase (EC 2.6.1.2), in the presence of catalytic amounts of pyridoxal phosphate as the coenzyme and α-ketoglutarate as the cosubstrate in D_2O as a cheap isotope source[11]. By repetition of the procedure we could enhance the deuterium degree up to > 99%. Esterification, N-formylation and oxazole ring closure[12] of the deuterated amino acid **5** give the labelled dienophile **6** in 70% overall yield.

Heating up the two Diels-Alder components **6** and **7** under various conditions, in the presence of radical inhibitors, predominantly gives nitrogen-free decomposition products, apparently due to the thermal instability of the unsaturated aldehyde **7**.

The corresponding dienophile with a protected aldehyde function, the relatively more stable alkoxy dihydrofuran **8**, which arises from THF[13,14], then indeed allows to carry out the cycloaddition reaction (50-60%). The desired electronic deactivation of the labilizing aldehyde function, however, simultaneously brings about an extensive electronic egalization of the two olefinic carbon atoms, and thus the loss of any regiocontrol in the Diels-Alder reaction: both possible regioisomers **10a** and **11** are formed in equal amounts (>98% D).

Surprisingly, the undesired product **11** can very smoothly be transformed into the natural, pyridoxal type isomer **10a** by treatment with acid. By this quantitative isomerization[15] our reaction sequence constitutes an attractive new pyridoxal synthesis - apart from its applicability to isotope labelling.

As the alkaloids we have detected in mammalians[5] cannot be analyzed mass spectrometrically in their endogeneous, phosphorylated form, not even by modern ionization techniques such as FD or FAB, they must be dephosphorylated enzymatically in the presence of a phosphatase (EC 3.1.3.2), beforehand. Thus, for our purpose the labelled pyridoxal **10b** does not have to be phosphorylated, but can directly be condensed with bi-nucleophiles like L-dopa and L-trypto-



Reagents and reaction conditions

1) glutamate pyruvate transaminase (EC 2.6.1.2), pyridoxal phosphate, α-keto-glutarate, D_2O, pD = 7, 38°C; 2) EtOH/HCl; 3) HCO_2H, HCO_2Na, Ac_2O, 5°C; 4) P_2O_5, MgO, Celite, $CHCl_3$, reflux; 5) 135°C, **8** twentyfold; 6) SiO_2; 7) EtOH/HCl; 8) 0.2N HCl, 35-42% from **4**; 9) H_2O, pH = 6, 38°C.

phan to yield the corresponding alkaloids **12** and **13** as internal standards for quantitative analyses.

Acknowledgement

This work was supported by the Minister für Wissenschaft und Forschung des Landes Nordrhein-Westfalen. One of us (S.S.) thanks the Fonds der Chemischen Industrie for generous fellowship support.

References and notes

1. "Endogene Alkaloide im Menschen, 3"; 2nd communication: lit.[5].
2. This work was presented in part at the 30th IUPAC congress in Manchester, UK, September 10, 1985, and at the 20. GDCh-Hauptversammlung in Heidelberg, FRG, September 16, 1985.
3. W. Whaley and T. Govindachari, Org. React. 6, 151 (1951).
4. M.A. Collins in The Alkaloids XXI (A. Brossi), S. 329, Academic Press, New York 1983;
   G. Bringmann, Naturwissenschaften 66, 22 (1979).
5. G. Bringmann and S. Schneider, Angew. Chem., submitted for publication.
6. H. Bader, Lehrbuch der Pharmakologie und Toxikologie, Edition Medizin, Weinheim 1983.
7. H. Groebe, Dtsch. med. Wschr. 98, 1313 (1973).
8. W.D. Lehmann and H.-R. Schulten, Angew. Chem. 90, 233 (1978).
9. S.P. Coburn, C.C. Lin, W.E. Schaltenbrand and J.D. Mahuren, J. Labelled Compd. Radiopharm. 19, 703 (1982); the yields for the single steps are: 42% (N-benzylation), 28% (hydrogen exchange and debenzylation), 26% (oxidation).
10. H. König and W. Böll, Chem.-Ztg. 100, 105 (1976).
11. T. Oshima and N. Tamiya, J. Biochem. 46, 1675 (1959).
12. Daiichi Seiyaku Co. (T. Naito, Y. Morita, S. Onishi and I. Tabara), Japan. 70 30,816 (October 6, 1970); Chem. Abstr. 74, P 42347 b (1971).
13. W. Reppe et al., Liebigs Ann. Chem. 596, 115 (1955).
14. F. Quennehen and H. Normant, Compte Rend. 228, 1301 (1949).
15. This isomerization reaction may be rationalized by elimination and regiospecific readdition of ethanol via a reactive furo[3,4-c]pyridine.

(Received in Germany 7 October 1985)