The alcohol **4**, $C_{15}H_{26}O$ (0.03%), was identified as cubenol by comparison of $[\alpha]_D$ and spectral data with those reported in the literature[5,7]. Compound **5** was isolated as an oil (0.08%), $[\alpha]_D = +15.4°$ (c 1 in EtOH). High resolution mass spectrometry established the elemental composition as $C_{15}H_{26}O$. The IR- and UV-spectra indicated the absence of hydroxyl or carbonyl group in the molecule. The single oxygen atom must be part of an ether bridge connecting 2 fully substituted carbon atoms; the IR-spectrum exhibited strong absorption at 1090 cm^{-1}, while the ^{13}C-NMR showed 2 singlets at 72.45 and 69.65 ppm. This spectrum also comprised 4 methines (47.27, 32.36, 30.89, 28.04), 5 methylenes (39.33, 34.80, 33.96, 27.31, 18.86) and 4 methyls (25.91, 22.29, 22.29, 21.34). The ^1H-NMR spectrum (270 MHz, $CDCl_3$) displayed singlets at δ 1.12 and 1.14 assignable to tertiary methyls attached to oxygen-bearing carbons. Other diagnostically valuable signals appeared at δ 0.69 (3H, d, J = 6.7 Hz) and 0.93 (3H, d, J = 6.7 Hz) and were assigned to methyls of an isopropyl group, since they collapsed to singlets by irradiation at frequency of an 1H multiplet at δ 1.73. From the above data it was established that **5** possessed the cadalane skeleton bearing an ether bridge connecting position 4 and 10. Closure of the oxane ring requires a cis relationship between H-1, H-6, Me-4 and Me-10. The relative stereochemistry of the remaining chiral centre at C-7 was established by the following criteria. a) Irradiation at δ 0.91 caused the isopropyl methine multiplet to collapse into a slightly broadened singlet; this revealed that the 1H signal masked by methyl groups, but evidenced by integration, was due to H-7. b) The signal of this proton and those of the isopropyl group remained almost unaffected by the addition of $Eu(fod)_3$ and thus the isopropyl-bearing ring must have, as expected, a chair conformation. c) In the ^1H-NMR spectrum a signal (dddd, J = 13.5, 13.5, 4.5, 4.5) is seen at δ 1.98, partially obscured by other protons, but well separated in C_6D_6; this signal, which suffers a remarkable europium shift, is simplified to a double-double doublet (J = 13.5, 13.5, 4.5) by irradiation at the frequency of H-7 (0.91); this result can only be explained assuming that the signal at δ 1.98 is due to the axial proton attached to C-8 and that H-7 is equatorial. Therefore, the new ether is 4,10-epoxymuurolane possessing the relative stereochemistry depicted in **5**. It is worth noting that *D. fasciola* accumulates sesquiterpenoids based on the cadalane skeleton, whereas the congener species *D. ligulatus* synthesizes perhydroazulene diterpenes[8].

Cadalane sesquiterpenes have been previously isolated from algae of the related genus *Dictyopteris* (cadalene, (−)-γ_1-cadinene and (−)-δ-cadinol from *D. divaricata*[9] and zonarene from *D. zonaroides*)[10].

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2 J.T. Baker and V. Murphy, Compound from marine organisms, vol. I. CRC Press, Cleveland 1976; P.J. Scheuer, Chemistry of marine natural products. Academic Press, New York 1973.
3 V. Amico, G. Oriente, M. Piattelli, C. Tringali, E. Fattorusso, S. Magno and L. Mayol, Experientia *33*, 989 (1977).
4 N.H. Andersen, D.D. Syzdal and C. Graham, Tetrahedron Lett. *1972*, 905.
5 B.A. Nagasampagi, L. Yankov and Sukh Dev, Tetrahedron Lett. *1968*, 1913.
6 S.J. Terhune, J.W. Hogg and B.M. Lawrence, Phytochemistry *13*, 1183 (1974).
7 Y. Ohta and Y. Hirose, Tetrahedron Lett. *1967*, 2073.
8 V. Amico, G. Oriente, M. Piattelli, C. Tringali, E. Fattorusso, S. Magno and L. Mayol, J. chem. Soc. chem. Commun. *1976*, 1069; B. Danise, L. Minale, R. Riccio, V. Amico, G. Oriente, M. Piattelli, C. Tringali, E. Fattorusso, S. Magno and L. Mayol, Experientia *33*, 413 (1977).
9 T. Irie, K. Yamamoto and T. Masamune, Bull. chem. Soc. Japan *37*, 1053 (1964).
10 W. Fenical, J.J. Sims, R.M. Wing and P.C. Radlick, Phytochemistry *11*, 1161 (1972).

Synthesis of 5'-deoxypyridoxal derivatives

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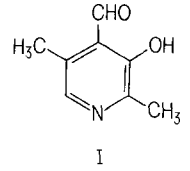
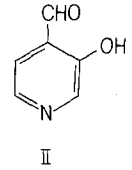
Summary. A convenient synthesis of 5'-deoxypyridoxal derivatives is described. The method involves catalytic hydrogenolysis of the corresponding 5'-phosphorylated derivatives; products are obtained in high yields.

5'-Deoxypyridoxal (**I**) and 3-hydroxypyridine-4-carboxaldehyde (**II**) are the best compounds available for the study of the mechanism of vitamin B_6 catalysis in model systems and have been used extensively for that purpose[3-11]. These vitamin analogs have the functional groups essential for pyridoxal phosphate-like catalysis and at the same time avoid the complications associated with pyridoxal phosphate and pyridoxal. In the case of pyridoxal phosphate, kinetic and equilibrium studies as a function of pH become more difficult owing to ionizations of the 5'-phosphate group. In the case of pyridoxal, internal hemiacetal formation between the 5-hydroxymethyl group and the 4-carboxyaldehyde group creates a condition that does not exist in the enzymically active, phosphorylated form of the vitamin[12]. Consequently, the vitamin analogs **I** and **II** are better in vitro model compounds than the naturally occuring forms of vitamin B_6. In addition to model system studies, compounds of this type have been used in the study of structure-activity relationships in vitamin B_6-dependent enzymes[13-16].



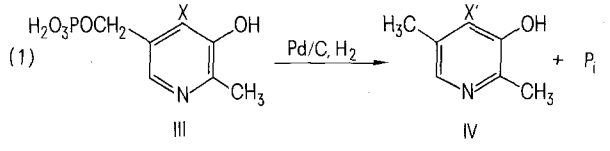
I



II

Several schemes for the synthesis of 5'-deoxypyridoxal, **I**[17-19], and 3-hydroxypyridine-4-carboxaldehyde, **II**[4, 20-22], have been reported. However, even the best synthetic approach for the preparation of 5'-deoxypyridoxal requires 5 steps[23], starting from commercially available pyridoxine. We report in this communication a general synthetic method that conveniently leads, in high yields, to the

production of 5'-deoxypyridoxal derivatives, using as starting materials the readily available corresponding phosphorylated compounds. The method involves, hydrogenolysis using 10% palladium on charcoal as catalyst. The 5'-phosphate ester group in **III** becomes reduced to the methyl group as shown in eq. 1, accompanied by the release of inorganic phosphate.



III IV

(X = CHO or CH_2NH_2) (X' = CH_2OH or CH_2NH_2)

Proof of structures was made by comparison with authentic samples of the products obtained after catalytic hydrogenolysis. In addition to the experiments described below, we also have been able to obtain by this method 5'-deoxypyridoxyl-amino acids from the corresponding 5'-phosphopyridoxyl derivatives.

Experimental. 5'-Deoxypyridoxamine (**IV**, X' = CH_2NH_2) dihydrochloride. Pyridoxamine phosphate monohydrochloride monohydrate (250 mg, 0.88 mmoles) was dissolved in 7 ml of water containing 150 mg of 10% palladium on charcoal. Hydrogen gas was continually passed through the well-stirred reaction mixture for 24 h at atmospheric pressure. The catalyst was filtered and the filtrate applied to a 1.8 × 20 cm column of Dowex 50 × 8 (50–100 mesh) in the hydrogen form. The column was washed with 500 ml of water, followed by 500 ml of 1 N HCl. The product was then eluted with 3.6 N HCl until the absorbancy at 295 nm dropped below 0.10; this required approximately 1.4 l. The solvent was removed on a rotary evaporator and the residual white solid was evaporated from ethanol and then ether. After drying to constant weight in a vacuum desiccator, the yield of 5'-deoxypyridoxamine · 2 HCl was found to be 177 mg (90%). This product was identical to an authentic sample[8] with respect to its NMR-, UV-, and mass spectra. On TLC, it migrated as a single spot having an R_f identical to that of an authentic marker sample in 2 different solvent systems.

5'-Deoxypyridoxine (**IV**, X' = CH_2OH) hydrochloride. Pyridoxal phosphate monohydrate (250 mg, 0.94 mmoles) was reduced to 5'-deoxypyridoxine as described above for the hydrogenolysis of pyridoxamine phosphate. After the catalyst was filtered, the pH of the filtrate was adjusted to approximately 6.5 with dilute KOH solution. The solvent was removed on a rotary evaporator and the residue was exhaustively extracted with hot ethanol. Evaporation of the ethanol left a white solid that was converted to 5'-deoxypyridoxine · HCl by evaporation from dilute HCl; the yield was 147 mg (82%). After recrystallization from ethanol/ether, the compound was shown to be identical to an authentic sample[23] of 5'-deoxypyridoxine · HCl by UV-, IR-, NMR-, and mass spectra, as well as by melting point and TLC. This procedure works equally well using glacial acetic acid as the solvent instead of water. Reoxidation of the 4-hydroxymethyl group to a 4-carboxaldehyde group can be readily accomplished using published procedures[19,23].

1 Supported by PHS Grant No. 5429-16-9.
2 To whom inquiries may be addressed.
3 T.C. Bruice and R.M. Topping, J. Am. chem. Soc. *85*, 1493 (1963).
4 T.C. French, D.S. Auld and T.C. Bruice, Biochemistry *4*, 77 (1965).
5 J.W. Thanassi, A.R. Butler and T.C. Bruice, Biochemistry *4*, 1463 (1965).
6 D.S. Auld and T.C. Bruice, J. Am. chem. Soc. *89*, 2098 (1967).
7 J.W. Thanassi, Biochemistry *9*, 525 (1970).
8 J.W. Thanassi, Biochemistry *11*, 2909 (1972).
9 J.W. Thanassi, Biochemistry *12*, 5109 (1973).
10 M. Blum, W.C. Cunningham and J.W. Thanassi, Bioorg. Chem. *5*, 415 (1976).
11 M. Blum and J.W. Thanassi, Bioorg. Chem. *6*, 31 (1977).
12 D. Heyl, E. Luz, S.A. Harris and K. Folkers, J. Am. chem. Soc. *73*, 3430 (1951).
13 J.C. Rabinowitz and E.E. Snell, Archs Biochem. Biophys. *43*, 408 (1953).
14 L.J. Arcement, W. Korytnyk and W.B. Dempsey, Bact. Proc. *68*, 121 (1968).
15 W.A. Newton, Y. Morino and E.E. Snell, J. biol. Chem. *240*, 1211 (1965).
16 J.E. Ayling and E.E. Snell, Biochemistry *7*, 1626 (1968).
17 D. Heyl, S.A. Harris and K. Folkers, J. Am. chem. Soc. *75*, 653 (1953).
18 T. Kuroda, Vitamin *29*, 116 (1964); Chem. Abstr. *62*, 515 (1965).
19 C. Iwata, Biochem. Prep. *12*, 117 (1965).
20 D. Heinert and A.E. Martell, Tetrahedron *3*, 49 (1958).
21 D. Heinert and A.E. Martell, J. Am. chem. Soc. *81*, 3933 (1959).
22 M.H. O'Leary and J.R. Payne, J. med. Chem. *14*, 773 (1971).
23 P.F. Mühlradt and E.E. Snell, J. med. Chem. *10*, 129 (1966).

Free amino acids in the adult citrus brown mite, *Eutetranychus orientalis* (Klein)

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Summary. Amino acids contained in extracts of adult *Eutetranychus orientalis* were separated and determined quantitatively by 2-dimensional paper chromatography. 14 amino acids were identified. Asparagine, ornithine, histidine, lysine, aspartic acid, serine and glycine were the major components of the free amino acid pool, comprising 83.94% of the total content.

Free amino acids in insect haemolymph and tissue have been frequently studied and reviewed[1–9]. Studies on the free amino acids in mites have not received considerable attention. The aim of the present study was to determine the concentration of the free amino acids in female adult citrus brown mite, *Eutetranychus orientalis* (Klein), which is considered as a noxious pest on citrus trees in Egypt[10,11].

Materials and methods. Female adults of the citrus brown mite, *E. orientalis*, were collected from a culture maintained on 1-year-old seedlings of sour orange. The procedure of Pant and Agrawal[12] was used for the preparation of amino acid extracts from adult citrus brown mite (about 500 mites). Free amino acids in the tissue extract were separated and determined quantitatively by 2-dimensional paper