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X=Y-ZH SYSTEMS AS POTENTIAL 1,3 - DIPOLES. PART 25. INTRAMOLECULAR CYCLOADDITION REACTIONS OF PYRIDOXAL IMINES OF ε-ALKENYL α-AMINO ESTERS. A POSSIBLE NEW APPROACH TO PYRIDOXAL ENZYME INHIBITION

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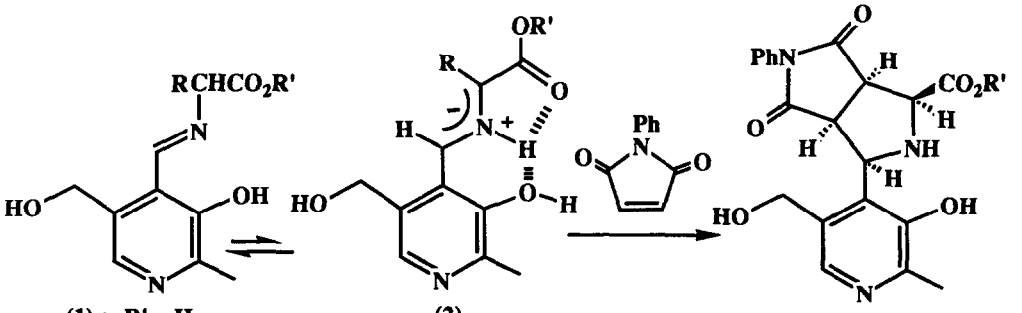
Abstract Pyridoxal imines of ε-alkenyl α-amino esters undergo an intramolecular cycloaddition, via an intermediate azomethine ylide, on keeping at room temperature for a prolonged period of time. The implications of this observation for the design of inhibitors of pyridoxal phosphate-dependent enzymes is discussed.

The potential of mechanism-based pyridoxal enzyme inhibitors as a source of novel drugs has focussed attention on this area in recent years. A range of novel β-substituted α-amino acids has been designed and synthesised, and evaluated *in vivo*. These xenobiotic β-substituted α-amino acids such as β-fluoroalanines, O-acetylserine, vinyl- and ethynyl-α-amino acids have been observed to inhibit pyridoxal phosphate-dependent racemases,[1] transaminases,[2] decarboxylases[3] and γ-lyases.[4] Elegant work by Metzler's group[2,3] uncovered the important, and unsuspected, mechanistic role of amino acrylic acid in the inhibition of pyridoxal enzymes by β-substituted alanine derivatives.

Our interest in pyridoxal chemistry arose from our demonstration that X= Ÿ-ZH systems undergo a facile thermal equilibration with their 1,3-dipolar tautomers X=Y(H)-Z, via 1,2 - prototropy.[5] Imines (X=C, Y=N, Z=CR) furnish particularly good examples of this prototropy and give rise to azomethine ylides. An important controlling factor in the ease of dipole formation is the pK_a of the ZH proton,[6] with electron withdrawing groups promoting dipole formation. Thus imines of α-amino acids[7] and their esters[8] are excellent precursors of azomethine ylides. Analogous processes occur in pyridoxal imines of α-amino acids (1a)[9] and their esters (1b)[10], and the azomethine ylides (2)[2] can be trapped in cycloaddition reactions (2) -> (3) with N-phenylmaleimide (NPM)

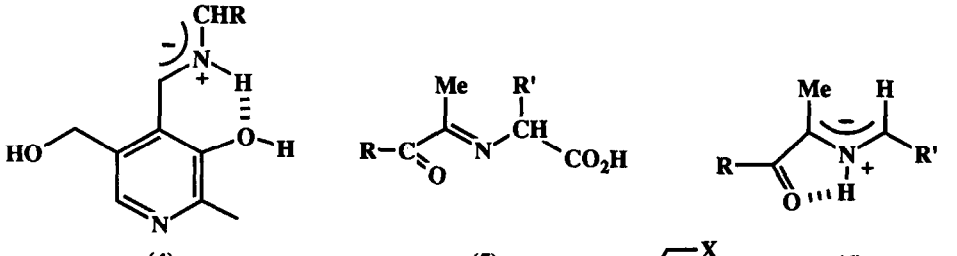
Subsequently we showed that related azomethine ylides (4) are generated in *in vitro* decarboxylation reactions of (1a).[9,12] Recently we have shown that pyruvate imines of α-amino acids (5, R=OH, Et or NH_2) undergo regiospecific decarboxylation generating azomethine ylides (6, R=OH, Et or NH_2) stereospecifically.[13] The formation of azomethine ylides (2), (4) and (6) suggest a novel approach to the design of suicide inhibitors of pyridoxal phosphate-dependent enzymes and pyruvate-dependent



(1) a. R' = H
b. R' = Me

(2)

(3)



(4)

(5)

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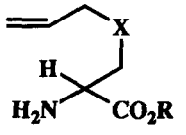
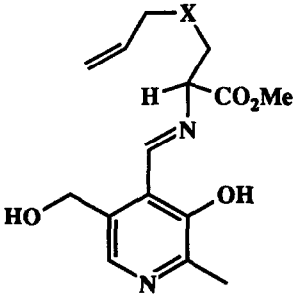
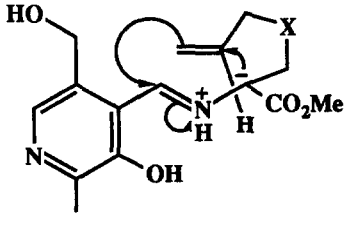


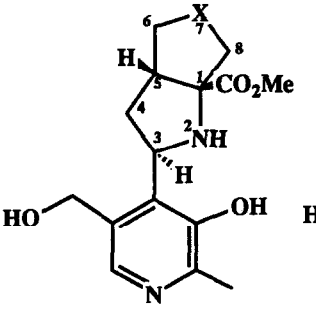
(7) a. R = Me, X = S
b. R = Me, X = CH_2
c. R = H , X = SO



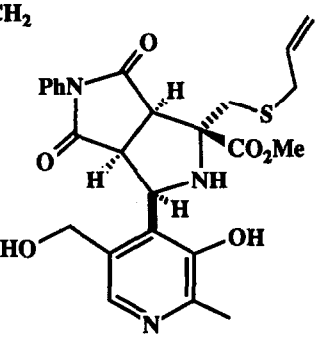
(8) a. X = S
b. X = CH_2



(9) a. X = S
b. X = CH_2



(10) a. X = S
b. X = CH_2

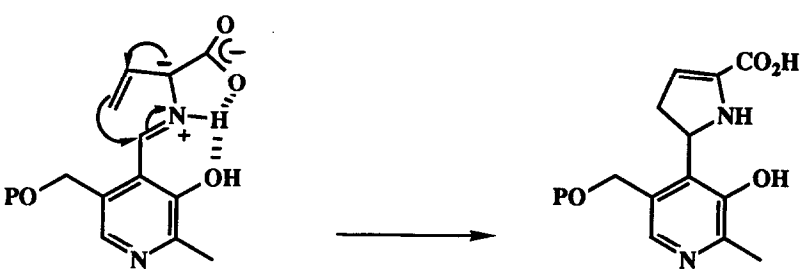


(11)

decarboxylases[14] by utilising α-amino acids incorporating a suitably positioned dipolarophile.[15]

α-Amino esters (7a) and (7b) were prepared by esterification of the known α-amino acids using thionyl chloride-methanol. The α-amino esters (7a) and (7b) reacted with pyridoxal to afford the imines (8a) and (8b) as viscous oils. When the imines (8a) and (8b) were heated in deuteroacetonitrile at 80°C for 18h in n.m.r. tube experiments, formation of the intramolecular cycloadducts (10a) and (10b) occurred (ca 20-30%) but was accompanied by extensive decomposition. However, the formation of (10) provided good circumstantial evidence for the intervention of dipole (9). When imine (8b) was reacted (MeCN, 80°C, 4h) with N-phenylmaleimide under similar conditions the intermolecular cycloadduct (11) was obtained in 58% yield. This suggests that the low yields of (10a) and (10b) are due the thermal instability of the imines (8a) and (8b) which manifests itself if the cycloaddition step is slow as in the case of the non-activated terminal alkene moiety in (9a) and (9b). However, the imines (8a) and (8b) solidified on keeping at room temperature for 3 months and examination of these solids showed them to be the desired intramolecular cycloadducts (10a) and (10b) respectively, which could be isolated in 80-100% yield. The stereochemistry of (10a), (10b), and (11) is assigned by analogy with that of previously described pyridoxal derived cycloadducts.[9,10]

The room temperature intramolecular cycloaddition (8) -> (10) suggests this approach could provide new types of suicide substrates for pyridoxal phosphate-dependent enzymes. It is, perhaps, of significance that (S)-(+)-allyl-L-cysteine sulphoxide (7) is found in garlic,[16] a plant renowned from ancient times for beneficial properties, and extracts of which have been shown to have antibacterial properties.[17] Such properties could arise from inhibition of alanine racemase[1] via a cycloaddition mechanism analogous to (8) -> (10). Moreover, dipole formation from pyridoxal imines offers an alternative mechanism for the known inhibition of pyridoxal enzymes by vinyl glycine. Thus, this inhibition might occur via a 1,5 - electrocyclisation (12) -> (13) analogous to that already observed by us for an arylidene imine of a vinyl α-amino ester.[15,18]



(12) P = PO_3^{2-} (13)

Experimental General spectroscopic details were as previously noted.[19]

S-(2-Propenyl)-L-cysteine methyl ester hydrochloride (7a, hydrochloride).

Thionyl chloride (7.14g, 4.35ml) was added over 5 min. to dry methanol (25ml) cooled to -10°C and the resulting solution stored for a further 5 min. when S-(2-propenyl)-L-cysteine (3.22g)[20] was added. The amino acid dissolved over ca. 5 min., and the resulting solution was stored at -10°C for 2h., kept at room temperature for a further 16h. and then poured into ether (600ml) and refrigerated for 2h. The product (3.25g, 77%) separated as colourless needles, m.p. 117°C, and was removed by filtration. (Found: C, 39.60; H, 6.95; N, 7.10. $C_7H_{14}ClNO_2S$ requires C, 39.70; H, 6.65; N, 6.60%). $\delta(D_2O)$ 5.75(m,1H,$\underline{CH}$=CH_2), 5.14(m,2H,CH=$\underline{CH_2}$), 4.29(dd,1H,$C\underline{H}CO_2Me$), 3.79(s,3H,OMe) and 3.02(m,4H, 2 x CH_2); ν_{max} 3300-2500(br), 1733, 1632 and 990cm^{-1}; m/z (%), 175(M-HCl,4), 116(17), 103(21), 88(100) and 74(43).

Methyl 2-aminohept-6-enoate hydrochloride (7b, hydrochloride) Prepared from the corresponding α-amino acid by the above method. After addition of ether and refrigeration the hydrochloride of (7b) was obtained as a colourless fluffy precipitate (49%) which was very hygroscopic and was used for imine formation without further purification. $\delta(D_2O)$ 5.81(M,1H,$\underline{CH}$=CH_2, 5.00(m,2H,CH=$\underline{CH_2}$); ν_{max} 3200-2500, 1735, 1585, 1000, 910 and 760cm^{-1} m/z(%) 157(M-HCl,1), 98(100), 88(20), 81(17) and 56(50).

Methyl N-pyridoxylidene 2-amino hept-6-enoate (8b). Pyridoxal hydrochloride (960mg, 4.73 mmol) and methyl 2-aminohept-6-enoate hydrochloride (916mg, 4.73 mmol) were dissolved in IN aqueous potassium hydroxide (9.5ml, 9.5 mmol). An immediate bright yellow colour developed and after stirring for 0.5h an orange oil had separated and was extracted with chloroform (2 x 25ml). The combined extracts were washed with water (2 x 25ml), dried ($MgSO_4$), and evaporated to yield a thick bright orange oil (830mg, 67%) which could not be distilled (Found: C, 62.45; H, 7.40; N, 9.25. $C_{16}H_{22}N_2O_4$ requires C, 62.70; H, 7.25; N, 9.15%); δ 9.10 (br s, 1H, ArOH), 8.91 (s, 1H, CH=N), 7.70 (s,1H,ArH), 5.7 (m,1H,$\underline{CH}$=CH_2), 4.98 (m, 2H CH=$\underline{CH_2}$) 4.73 (s, 2H, CH_2), 2.46(s, 3H, Me) and 2.13-1.37 (m, 6H, 3 x $4CH_2$); ν_{max} 3600-3000(br), 1730, 1620, 1390, 1205, 1022, and 910cm^{-1}; m/z(%) 306 (M^+, 35), 247(19), 165(52), 150(30), 149(38), 98(100), 88(19), 81(28) and 56(43).

Methyl N-pyridoxylidene S-(2-propenyl)-L-cysteine(8a). Prepared from pyridoxal hydrochloride and S -(2-propenyl)-L-cysteine methyl ester hydrochloride in a manner analogous to that described above. The product (89%) was a thick yellow oil with a garlic odour which could not be distilled. Accurate mass: 324.1142. $C_{15}H_{20}N_2O_4S$ requires 324.1143; δ 9.50(br s, 1H, ArOH), 8.86 (s, 1H, CH=N), 7.61(s, 1H, ArH), 5.66(m, 1H, $\underline{CH}$=CH_2), 5.04(m, 2H, CH=$\underline{CH_2}$), 4.67(s, 2H, $\underline{CH_2}$OH), 4.3(br s, 1H, $CH_2C\underline{H}$), 4.11(dd, 1H, $\underline{CH}CO_2Me$), 3.72 (s, 3H, OMe), 3.10-2.79 (m, 4H, 2 x CH_2S) and 2.39 (s, 3H, Me); ν_{max} 3600-3000(br), 1737, 1628, 1395m 1210, 990 and 920cm^{-1}; m/z(%) 324(M^+,6), 265(1), 150(17), 149(12), 103(9), 74(100) and 41(67).

Methyl3-(3^1-hydroxy-5^1-hydroxymethyl-2^1-methyl-4^1-pyridyl)-2-aza-7-thiabicyclo[3.3.0]octane-2-carboxylate (10a). After standing in a stoppered flask for ca. 3 months at room temperature imine (8a) was seen to have solidified. The n.m.r. spectrum of this solid showed to it consist of pure (10a). The solid was crystallised from methanol to afford yellow prisms, m.p. 205°C (Found: C, 55.25; H, 6.45; N, 8.30; S, 9.75. $C_{15}H_{20}N_2O_4S$ requires C, 55.55; H, 6.20; N, 8.65; S, 9.90%); $\delta(CD_3CN)$ 9.75(s, 1H, ArOH), 7.69 (s, 1H, ArH), 5.02(dd, 1H, 3-H), 4.44(s, 2H, $\underline{CH_2}$OH), 3.69 (s, 3H, OMe), 3.47(d, 1H, 8-H), 3.18 (m, 1H,

5-H), 2.24(s, 3H, Me) and 2.05(M, 2H, 2 x 4-H); ν_{max} 3600-3000(br), 3240, 1732, 1415, 1245 and 1040cm^{-1}; m/z(%) 324(M^+, 54), 306(24), 265(17), 259(20), 247(39), 192(32) and 118(57).

Methyl 3-(3^{I}-hydroxy -5^{I}- hydroxymethyl -2^{I}- methyl -4^{I}- pyridyl) -2- azabicyclo [3.3.0] octane -2-carboxylate (10b). After standing in a stopped flask for ca. 3 months imine (8b) (560mg) was seen to have solidified. After washing with ether the product (450mg, 80%) was obtained as an amorphous yellow solid, m.p. 130-135°C. Crystallisation from ether -methanol afforded pale yellow prisms, m.p. 138-140°C (Found: C, 62.40; H, 7.55; N, 8.95. $C_{16}H_{22}N_2O_4$ requires C, 62.75; H, 7.25; N, 9.15%); δ(CD_3CN) 7.64 (s, 1H, ArH), 4.73(dd, 1H, 3H), 4.44 (s, 3H, Me) and 2.10-1.40 (m, 8H, 4 x CH_2); ν_{max} 3600-3000(br), 3320, 1730, 1419, 1235, 1030, and 800cm^{-1}; m/z(%) 306(M^+, 70), 247(84), 229(100), 165(17), 164(20), 151(17), 98(38), 81(24) and 55(31).

Methyl 2-allylthiomethyl -4- (3^{I} - hydroxy -5^{I}- hydroxymethyl -2^{I}- methyl -4^{I}- pyridyl) -7- phenyl -6,8-dioxo -3,7 - diazabicyclo [3.3.0] octane -2- carboxylate (11). Methyl N-pyridoxylidene S-(2-propenyl) - L- cysteine (700mg, 2.16 mmol) and N-phenylmaleimide (370mg, 2.16 mmol) were boiled under reflux in acetonitrile (25ml) for 4h. The solvent was then removed under reduced pressure and the residual gum triturated with 40-60°C petroleum ether - ether to afford the product (620mg, 58%) as a colourless solid which crystallised from ether-methanol as colourless needles, m.p. 119-121°C (Found: C, 59.50; H, 5.45; N, 8.45. $C_{25}H_{27}N_3O_6S$ requires C, 60.35; H, 5.45; N, 8.45%); δ 10.80 (br s, 1H, PyrOH), 7.80 (s, 1H, PyrH), 7.45-7.11 (m, 5H, ArH), 5.80 (m, 1H, $\underline{CH}$=CH_2), 5.20 (m, 2H, CH=$\underline{CH_2}$), 5.09 (d, 1H, 4-H), 4.57 (m, 2H CH_2 OH), 4.05 (dd, 1H, 5-H), 3.87 (s, 3H, OMe), 3.50 (br s, 1H, NH), 3.54 and 2.82 (2 x d, 2H, SCH_2), 3.52 (d, 1H, 1-H), 3.16 (d, 2H, $S\underline{CH_2}$ CH=CH_2) and 2.42 (s, 3H, Me); ,ν_{max} 3600-3000(br), 3270, 1730, 1702, 1381, 1200, 930 and 691cm^{-1}; m/z(%) 497 (M^+, 4), 410(6), 392(40), 261(14), 247(10), 175(64), 173(85), 119(28), 74(66), 59(32) and 41(100).

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