Condensation of Pyridoxal with the Methyl Ester of Glycine (1,2)

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Condensation of pyridoxal with the methyl ester of glycine gave the aminocoumarin analog 3-amino-8-methyl-2-oxo-2*H*-pyrano[2,3-c]pyridine-5-methanol (III), and not the expected aldimine. The structure of the compound was deduced by independent synthesis and by comparing its NMR spectrum with that of 3-aminocoumarin. The new reaction provides routes for the synthesis of α^4-pyridoxylideneglycine and α^4-pyridoxyloxoacetic acid derivatives. It may also provide a model for irreversible inactivation of vitamin B_6 in biochemical systems.

Interactions of amino acids with pyridoxal (I) or its analogs have been the subject of intensive investigations for a number of years since they provide model systems for enzymatic reactions catalyzed by many enzymes containing pyridoxal phosphate (3). Generally the first step in these reactions is the formation of aldimines (II), which have been isolated in a few cases (4). Although most of these reactions have been carried out in water in the presence of appropriate metal ions, they were also found to take place in such relatively non-polar solvents as absolute ethanol (5). Esters of amino acids were also found to react in water but without metal ion catalysis (6).

In connection with a different problem, we investigated the reaction of pyridoxal with the methyl ester of glycine in pyridine as the solvent. After a reaction mixture consisting of equimolar amounts of reagents was stirred for about 15 minutes at 70°, a new compound started to precipitate. The yield was increased to 63% after prolonged stirring. The new compound proved to be stable to acid and decomposed about 400°, thus indicating that its structure could not be that of an aldimine (II), which is rather labile. The structure of the compound has been shown to be III on the basis of the following evidence.

2-(α^4-Hydroxyl-α^4-pyridoxyl)glycine (IV; "β-pyridoxylserine") has been prepared by treating the pyridoxal-glycine-Al^{+++} complex with acid, as described by Metzler, Longenecker, and Snell (7). The structure of this compound has now been confirmed by NMR spectroscopy (Table I) (8). Dehydration of IV with *N*,*N*′-dicyclohexylcarbodiimide in pyridine gave III as the only product.

Another confirmation of the structure was provided by comparison (primarily by NMR, Table I) with 3-aminocoumarin (VIa), which was first obtained by Linch as an *N*-acetyl derivative by the condensation of salicylaldehyde (V) with glycine in the presence of sodium acetate and acetic anhydride (9a); the parent compound was obtained by deacetylation (9b).

Acetylation of III gave the diacetyl derivative VII, which could be selectively deacetylated to yield the *N*-acetyl derivative VIII. Treatment of VIII with concentrated ammonia caused the lactone ring to open, giving *N*-acetyl-2-(α^4-pyridoxylidene)glycine (IX), without any appreciable *N*-deacetylation.

Linch interpreted several reactions of his 3-aminocoumarin (VI) as indicating the presence of a 3-imino tautomer, VIb (9a). NMR spectra of both 3-aminocoumarin (VI) and the pyridoxal reaction product III and their acetyl derivatives indicate only the α-vinyl proton; no indication of the methylene α^4-protons could be found in either the parent compounds or their derivatives (Table I). Some of the reactions of 3-aminocoumarin, such as the ease with which it deaminates, can be readily interpreted as being due to the enamine nature of the system (10). As expected, compound III also deaminates readily with 1 *N* sodium hydroxide in the cold or with boiling water, yielding the keto acid 2-(α^4-pyridoxyl)-2-oxoacetic acid (X). The latter readily forms a lactone (XI), which acetylates at the α^5-*O*-position, as indicated by the nmr spectrum in DMSO. A singlet due to the α^5-methylene group is expected in the case of a substituted α^5-OH group (11).

Compounds X-XII could exist in either the keto or enol forms. The keto acid X was found to exist in the keto form in an alkaline solution, and its lactone XI was found to exist in the enol form in an acid solution (Table I). The α^4-protons in these two compounds were found to be readily exchangeable with deuterons in base and in acid, respectively. The acetylated lactone (XII) and 3-hydroxycoumarin exist in the enol form in DMSO solution. Limited solubility of these compounds precluded a systematic study of their tautomerism.

Although the methylene group in glycine was found to be somewhat activated as it enters into reactions with carbonyl compounds (if appropriately catalyzed) (12), a reaction with pyridoxal under mild conditions resembling

SCHEME I

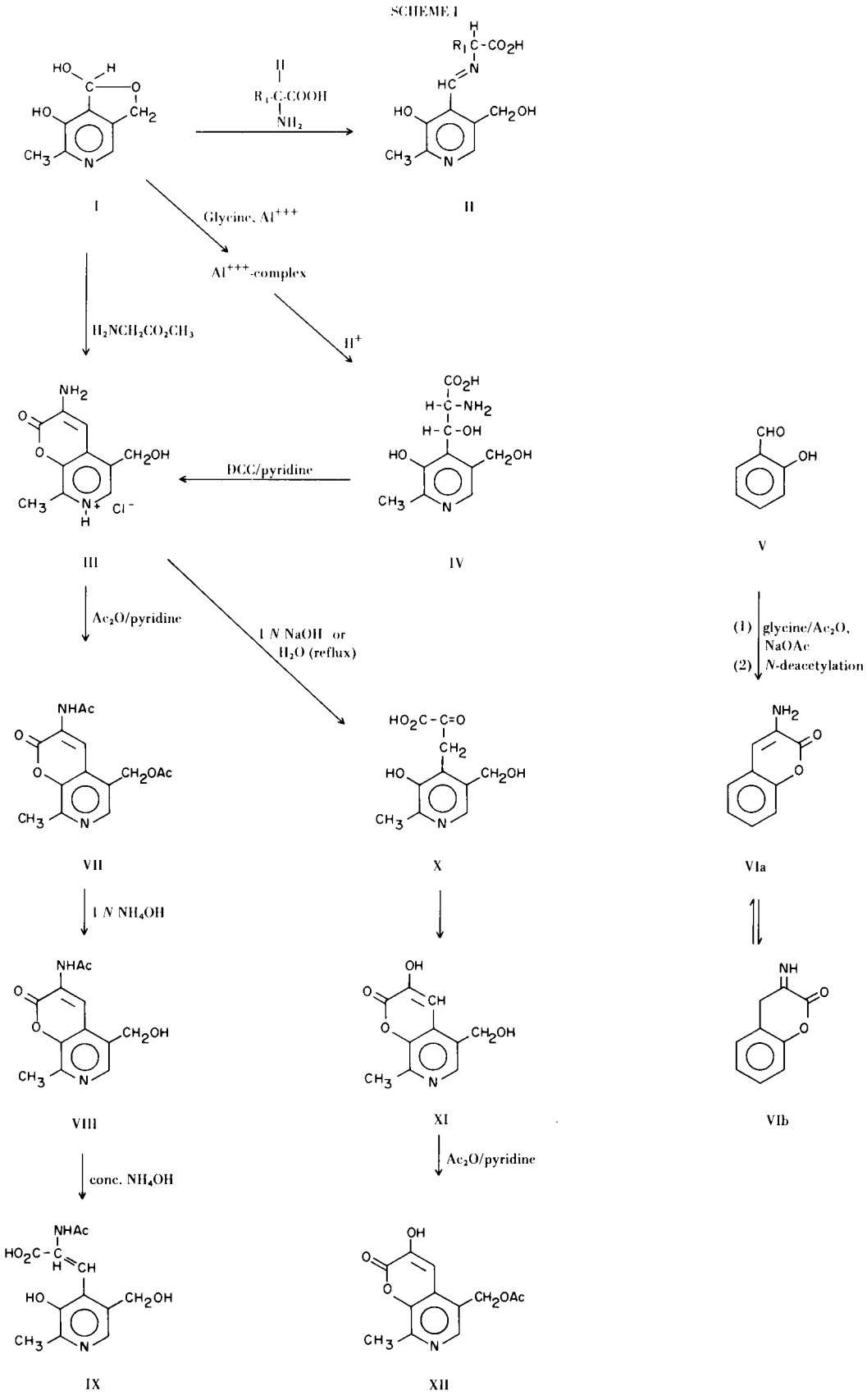
I
II
Glycine, Al+++
Al+++-complex
H+
H2NCH2CO2CH3
III
IV
DCC/pyridine
V
Ac2O/pyridine
1 N NaOH or H2O (reflux)
(1) glycine/Ac2O, NaOAc
(2) N-deacetylation
VII
X
VIa
1 N NH4OH
VIII
XI
VIb
conc. NH4OH
Ac2O/pyridine
IX
XII

TABLE I

Nuclear Magnetic Resonance Spectra (a)

Compound	Solvent	Pyr-CH_3 (b)	Pyr-CH_2OH (b)	Pyr α-CH or Phen α-CH (b)	Pyr-H or Phen-H (b)	Pyr β-CH or Phen β-CH (b)	Others
III	D_2O	162 (3)	287 (2)	402 (1)	487 (1)	----	
VII	$CDCl_3$	160 (3)	316.5 (2)	527.5 (1)	501 (1)	----	125 and 136.5 (acetyl CH_3); 496 (N-*H*)
VI	$CDCl_3$	---	---	398 (1)	430 (4)	----	242 (NH_2)
VI-*N*-acetyl	$CDCl_3$	---	---	521 (1)	435-458 (m, 4)	----	135 (*N*-acetyl CH_3) 389.5 (N*H*)
VIII	1 *N* D_2SO_4	173 (3)	300 (2)	525 (1)	508 (1)	----	140.5 (*N*-acetyl CH_3)
IX	D_2O	158 (3)	281.5 (2)	418 (1)	488 (1)	---	120 (*N*-acetyl CH_3)
IV	D_2O	161 (3)	291.5 (2)	345.5 (2, d, J = 7)	495.5 (1)	275.5 (2, d, J = 7)	
X	0.5 *N* NaOD	138 (3)	288 (2)	175 (2) (c)	444 (1)	----	
XI	1 *N* DCl	172 (3)	300 (2)	441 (1) (d)	507.5 (1)	----	
3-Hydroxycoumarin	DMSO-d_6	---	---	426 (1)	431 to 458 (4)	---	618 (br., O*H*)
XII	DMSO-d_6	159 (3)	325 (2)	443 (1)	511.5	----	122 (*O*-acetyl CH_3)

(a) Expressed in Hz at 60 MHz from TMS in organic solvents or from sodium 3-(trimethylsilyl)-1-propanesulfonate in aqueous solvents. Figures in parentheses refer to the number of protons. Abbreviations: d = doublet, m = multiplet. (b) "Pyr-" and "Phen-" refer to the pyridoxoid and phenyl analog compounds, respectively. (c) Determined in nondeuterated 0.5 *N* NaOH. In deuterated alkaline solution, a rapid exchange takes place. A similar exchange has been observed for phenylpyruvic acid, for which the position for the α-CH_2 peak has been determined at 200 cps in 0.5 *N* NaOH. (d) After standing for 60 hours, this peak decreased to 5% of its original area.

those used in the Knoevenagel condensation was rather unexpected. In order to get some insight into the reaction mechanism, we have been investigating the interaction of pyridoxal with some related compounds.

Glycine did not react with pyridoxal under these conditions, indicating that the methoxy group in the methyl ester of glycine is important as a leaving group. The methyl ester of sarcosine (*N*-methylglycine) also did not react, even when more vigorous conditions were used, indicating that the primary amino group is important. Similarly, little reaction was detected with diethyl malonate, which has a much more activated methylene group.

Since pyridoxal in the hemiacetal form has a "masked" aldehyde group, aldimine formation (II, R_1 = H) with the methyl ester of glycine may be essential for activation of the α^4-carbon of pyridoxal, and may precede the attack by the weakly nucleophilic methylene group of the ester. Although the lack of reactivity of the methyl ester of *N*-methyl glycine is consistent with this idea, our evidence is not sufficient to permit any firm conclusions.

The reactions described afford a method for obtaining stable analogs of pyridoxal modified in the α^4-position by a direct reaction with a readily available compound. An indirect but more general method has been described previously by us (1a,13). Condensation of pyridoxal with the methyl ester of glycine under mild conditions provides an example of an irreversible inactivation of pyridoxal (or its 5′-phosphate) by the formation of a stable derivative (14). This reaction may provide a model for such inactivation in biochemical situations (15).

EXPERIMENTAL

Thin-layer chromatography was carried out on plates coated with silica gel as described earlier (1b). The most useful solvents for development were 1:1 chloroform-methanol, 1:1 ethanol-water, and 7:3 1-propanol-water. IR spectra were determined with a Perkin-Elmer 137B or 457 spectrometer, UV spectra with a Perkin-Elmer 202 spectrometer, and NMR spectra with a Varian A-60A instrument.

3-Acetylaminocoumarin was prepared by the method of Linch (9a), 3-aminocoumarin by that of Reppel and Schmollack (9b), and 3-hydroxycoumarin by that of Shaw *et al.* (16).

Reaction of Pyridoxal with the Methyl Ester of Glycine: 3-Amino-8-methyl-2-oxo-2*H*-pyrano[2,3-*c*]pyridine-5-methanol (III).

Pyridoxal hydrochloride (1.0 g., 4.9 mmoles) and the hydrochloride of the methyl ester of glycine (0.62 g., 5.0 mmoles) in pyridine (dry, 20 ml.) were stirred at 70° with moisture excluded. Precipitation started after 15 minutes. The mixture was kept at 50° for 6 hours and at room temperature overnight. Filtration and washing (1:1 ethanol-ether) yielded 750 mg. of needles (63%), which decomposed above 400°. λ max (ethanol) 262 mμ (ε 7,350), 370 mμ (ε 19,000); λ max (Nujol) 3300, 2125, 2600, 1725, 1615, 1570 cm^{-1}.

Anal. Calcd. for $C_{10}H_{11}ClN_2O_3$: C, 49.50; H, 4.57; N, 11.54; Cl, 14.61. Found: C, 49.44; H, 4.70; N, 11.46; Cl, 14.56.

Compound III from 2-(α^4-Hydroxyl-α^4-pyridoxyl)glycine (IV).

To a solution of IV (41 mg.) in dry pyridine (1.5 ml.), *N*,*N*′-dicyclohexylcarbodiimide (100 mg.) was added, and the mixture was stirred at room temperature for 24 hours. The residue was evaporated, and then was dissolved in ethanol (25 ml.) to which ethanolic hydrogen chloride (0.4 ml. of a 10% solution) had been added. The solution was heated to boiling and cooled, yielding 17.7 mg. (50%) of III, identical in all respects (TLC, UV, IR, and NMR) with the authentic material already described.

Acetylation of III: 5-Acetoxymethyl-3-acetamido-8-methyl-2-oxo-2*H*-pyrano[2,3-*c*]pyridine (VII).

To an ice-cooled solution of III (49 mg.) in pyridine (dry, 10 ml.), acetic anhydride (0.2 ml.) was added. The suspension was stirred for 4 hours, the clear solution was evaporated to less than 1 ml., and water added drop by drop until the acetate (VII) crystallized. After washing (cold ethanol) and drying, the yield amounted to 48 mg. (85%), m.p. 190-191° (after recrystallization from a mixture of ethanol and ethyl methyl ketone). λ max (ethanol) 243 mμ (ε 19,200), 310 mμ (ε 18,600); λ max (Nujol) 3310 cm^{-1} (NH), 1720 and 1690 cm^{-1} (*O*- and *N*-acetyl).

Anal. Calcd. for $C_{14}H_{14}N_2O_5$: C, 57.93; H, 4.86; N, 9.65. Found: C, 57.96; H, 4.76; N, 9.81. Molecular weight: Calcd. 290. Found (mass spectrum) 290.

Selective Deacetylation of VII: (a) 3-Acetamido-8-methyl-2-oxo-2*H*-pyrano[2,3-*c*]pyridine-5-methanol (VIII).

The diacetate (VII, 200 mg.) was dissolved in 1 *N* ammonium hydroxide (15 ml.) and stirred in darkness at room temperature for 18 hours, evaporated to about 5 ml., and allowed to crystallize in the cold. The yield was 60 mg. (35%), m.p. 295° (from ethanol). UV: λ max (ethanol) 233 mμ (ε 10,300), 310 mμ (ε 18,700); IR: λ max (Nujol) 3345 and 3195 cm^{-1} (C=O).

Anal. Calcd. for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.28. Found: C, 57.89; H, 4.87; N, 11.42.

N-Acetyl-2-(α^4-pyridoxylidene)glycine (IX).

The diacetate (VII, 48 mg.) was stirred with concentrated ammonia (10 ml.) for 60 hours. The clear solution was evaporated, and was crystallized from acetone, yielding IX (40 mg., 91%), m.p. 228° (from water-dioxane). UV: λ max (0.1 *N* hydrochloric acid) 299 mμ (ε 9,800); λ (0.1 *N* sodium hydroxide) 236 mμ (ε 17,900), 271 mμ (ε 8,400) 344 mμ (ε 7,500). IR: λ max (Nujol) 3500-2500 cm^{-1} (broad, OH bonded), 1670, 1625, 1570, 1560 cm^{-1} (broad peaks, C=O, C=N, and C=C).

This compound could also be obtained from the monoacetate (VIII) by the same method.

Anal. Calcd. for $C_{12}H_{14}N_2O_5$: C, 54.13; H, 5.30; N, 10.52. Found: C, 54.39; H, 5.31; N, 10.79.

Deamination of III: 2-(α^4-Pyridoxyl)-2-oxoacetic Acid (X).

The amine III (70 mg.) was refluxed in water (5 ml.) for 2 hours. A small amount of precipitate was filtered off, and the filtrate was concentrated to 2 ml. Acetone (2 ml.) was added, and the reaction mixture was cooled. The yield was 31 mg. (48%). At 300° decomposition occurs, and an olive-colored product sublimes. UV: λ max (0.1 *N* hydrochloric acid) 284 mμ (ε 3,700); λ max (0.1 *N* sodium hydroxide) 245 mμ (ε 8,600), 298 mμ (ε 8,770). IR: λ max (potassium bromide) 3650-2450 cm^{-1} (broad, OH-bonded), 1600 cm^{-1} (broad, C=C).

Anal. Calcd. for $C_{10}H_{11}NO_5$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.20; H, 5.01; N, 6.29.

2-(α^4-Pyridoxyl)-2-oxoacetic Acid δ-Lactone (XI).

Compound III (637 mg.) was dissolved in a minimum amount of 0.2 *N* sodium hydroxide, kept at room temperature for 10 hours, and neutralized with 5 *N* hydrochloric acid to *p*H 7.5. The precipitated compound XI was filtered and washed with water. Additional XI was obtained by heating the mother liquors to 100°, thus inducing more lactonization of X, which is more water-soluble. The total yield was 545 mg. (84%). The compound was recrystallized from diethylene glycol monoethyl ether; it slowly decomposed above 300°. IR: λ max (Nujol) 1730 cm^{-1} (very broad).

Anal. Calcd. for $C_{10}H_9NO_4$: C, 57.97; H, 4.38; N, 6.76. Found: C, 57.69; H, 4.52; N, 6.82.

2-[α^4-(α^5-*O*-Acetylpyridoxyl)]-2-oxoacetic Acid δ-Lactone (XII).

The lactone XI (45 mg.) was stirred overnight with pyridine (6.0 ml.) and acetic anhydride (0.35 ml.). A 5% sodium bicarbonate solution (5 ml.) was added and the yellow crystals that precipitated (40 mg., 74%) were collected, washed with water, and dried. The compound decomposed slowly above 400°. UV: λ max (DMSO) 296 mμ (ε 13,300); IR: λ max (potassium bromide) 1730 cm^{-1} (C=O of *O*-acetyl).

Anal. Calcd. for $C_{12}H_{11}NO_5$: C, 57.83; H, 4.45; N, 5.62. Found: C, 57.83; H, 4.63; N, 6.13.

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