SYNTHESIS AND PROPERTIES OF ANALOGS OF PYRIDOXAL

V. L. Florent'ev, N. A. Drobinskaya, L. V. Ionova, and M. Ya. Karpeiskii

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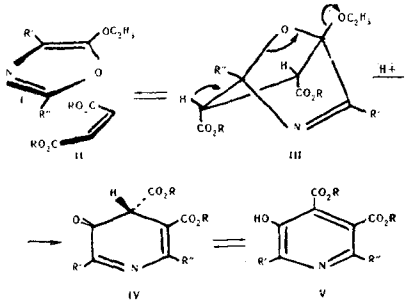
A method for the synthesis of analogs of pyridoxal—2-norpyridoxal, 6-methyl-2,4-pyridoxal, and 6-methylpyridoxal—has been worked out. The reaction of 5-ethoxyoxazoles with maleic diesters gave diesters of substituted 3-hydroxycinchomeric acids, which were converted by reduction with lithium aluminum hydride into analogs of pyridoxine. The latter were oxidized with magnesium dioxide to the corresponding analogs of pyridoxal. The oximes of the aldehydes and their Schiff's bases with p-phenetidine have been obtained. Analogs of pyridoxamine have been obtained by the hydrogenation of the oximes. The UV absorption spectra of the compounds and the ratios of the ionic forms in aqueous solutions have been studied.

Enzymes the cofactor of which is pyridoxal 5'-phosphate (PLP) occupy a key position in the metabolism of the amino acids [1, 2]. At the present time considerable progress is being achieved in understanding the mechanism of a number of reactions catalyzing this group of enzymes. Essential information on the structure of the active center of the PLP enzymes and, in particular, on the nature of the bond of the coenzyme with the apoenzyme can be obtained by studying both analogs of the enzyme itself and of its nonphosphorylated derivatives.

This paper is devoted to a description of the synthesis and some of the properties of nonphosphorylated analogs of PLP—2-norpyridoxal, 6-methylpyridoxal, and 6-methyl-2-norpyridoxal.

The diene condensation of 5-alkoxyoxazoles with various dienophiles must be regarded as the most general method for the synthesis of substituted 3-hydroxypyridines [3, 4]. This applies, in particular, to the preparation of B_6 analogs modified at positions 2 and 6, the synthesis of which is extremely difficult to effect by other methods.

Consequently, to obtain the above-mentioned analogs of pyridoxal we selected a route based on the diene condensation of 5-ethoxyoxazoles (I). The latter were synthesized by cyclizing esters of the corresponding N-acyl amino acids in the presence of phosphorus pentoxide [5, 6].

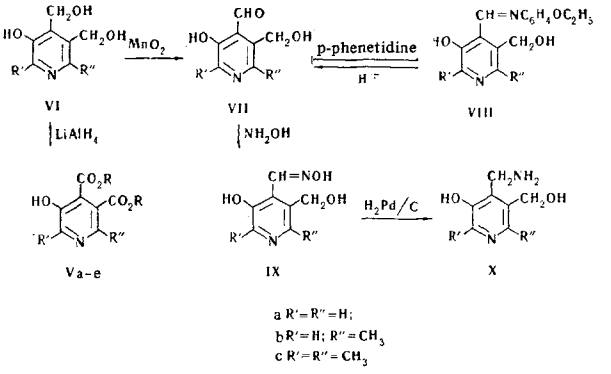


a R = CH_3; R' = R'' = H; b R = CH_3; R' = H, R'' = CH_3; c R = C_2H_5; R' = H; R'' = CH_3;
d R = CH_3; R' = R'' = CH_3; e R = C_2H_5; R' = R'' = CH_3

In the reaction of I with dimethyl or diethyl maleate (II), the adduct III is formed as an intermediate and this is then aromatized by the addition of a solution of hydrogen chloride in absolute ethanol, giving the diester of a substituted 3-hydroxycinchomeronic acid (V).

The $C_{(6)}$—O bond is loosened by conjugation with the —N=C— bond, which facilitates its heterolysis. In this process, the oxygen displaces the liberated electron pair of the ethoxide anion. This scheme assumes a hindered aromatization of the 6-alkyl-substituted adducts. In actual fact, the diene synthesis using 2-methyl- and 2,4-dimethyloxazoles takes place with lower yields than that using 2-unsubstituted oxazoles. A similar point of view on the mechanism of the diene synthesis of oxazoles has been put forward in a recent paper [7].

Below we show the further conversions of V into derivatives of pyridoxine, pyridoxamine, and pyridoxal.



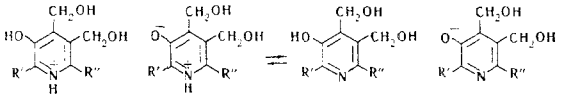
a R' = R'' = H;
b R' = H; R'' = CH_3
c R' = R'' = CH_3

The pyridoxine analogs VIa–VIc were obtained by lithium aluminum hydroxide reduction.

The selective oxidation of the 4-hydroxymethyl group of the diol VI was effected by the reaction of manganese dioxide in dilute sulfuric acid. The aldehydes VIII, pyridoxal analogs, were isolated from the reaction mixture either in the form of the oximes IX or in the form of the Schiff's bases VIII with p-phenetidine, the yields of the derivatives amounting to 66–79% calculated on the diol, except for the 6-methyl-2-nor analog.

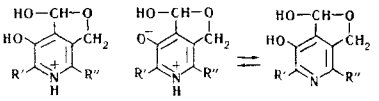
A convenient method of obtaining aldehydes is the hydrolysis of Schiff's bases during their chromatography on a sulfonic acid resin. Under these conditions it is easy to separate the p-phenetidine and to isolate the aldehyde VII in the pure form. The pyridoxamine analogs Xa–Xc were obtained by the catalytic hydrogenation of the oximes IX on Pd/C.

A study of UV spectra has shown that the properties of the compounds obtained depend to a considerable extent on the position of the methyl group.

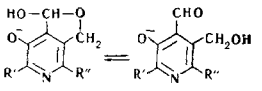


XI XII XIII XIV

The absence of a methyl group in position 2 of the pyridine ring leads to a shift in the equilibrium (**XII** ⇌ ⇌ **XIII**) in the direction of the neutral form **XIII** (Fig. 1).

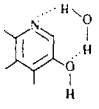


XV XVI XVII

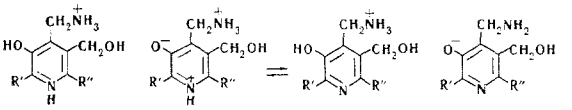


XVIII XIX

As can be seen from Table 1, for all the 2-nor derivatives the absorption maximum of the neutral form appears in an aqueous medium at pH 7. This is probably explained by the fact that in a substituted 3-hydroxypyridine with a free position 2 the neutral form is stabilized by the formation of a cyclic hydrate complex:



The introduction of a methyl group into position 2 makes this type of hydration impossible.

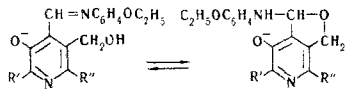


XX XXI XXII XXIII

The presence or absence of a methyl group in position 6 of the pyridine ring has no effect on the ratio of the ionic forms in a neutral medium.

At the same time, a 6-methyl group substantially affects the properties of the 4-formyl group of the pyridoxal analogs. Thus, in the spectra of 6-methyl-2-norpyridoxal and 6-methylpyridoxal the absorption maximum at 390 nm corresponding to the free aldehyde form **XIX** is completely absent (Fig. 2). A similar conclusion may be drawn from the measurement of the absorption of the aldehydes in phenylhydrazine at 410 nm [9]. The absorption of the phenylhydrazones of pyridoxal and 2-norpyridoxal reaches a maximum after heating at 60° C for 20 min (ε = 22,000 and 21,700, respectively). In the case of 6-methyl-2-norpyridoxal and 6-methylpyridoxal, the molecular extinctions amount to only 10,000-12,000 even after heating for 2 hr.

An analogous situation is found in the spectra of the Schiff's bases (see Fig. 3).



XXIV a XXIV b

The absorption of the nonacetalated imine form **XXIVa** at 390 nm is much lower for the 6-methyl derivatives.

The explanation of this phenomenon must probably be sought in the steric influence of the methyl group in position 6 of the pyridine ring. Rotation round the $C_{(5)}$—$C'_{(5)}$ bond is appreciably hindered and the conformation of the 5'-hydroxy group favors nucleophilic attack on the 4-aldehyde group. The high degree of acetalation of 6-methyl-2-norpyridoxal substantially interferes with the preparation of derivatives. Thus, the Schiff's base with p-phenetidine can be obtained only on heating and with a yield of only 38%.

EXPERIMENTAL

5-Ethoxyoxazole (I). With stirring, a solution of 65.5 g (57.5 ml; 0.5 mole) of the ethyl ester of N-formylglycine in 200 ml of dry $CHCl_3$ was added to a suspension of 142 g (1 mole) of P_2O_5 in 300 ml of ethanol-free $CHCl_3$. The mixture was heated in the water bath for 4 hr and was then cooled and, with vigorous stirring, 750 ml of 20% KOH solution was added. The chloroform layer was separated off, and the aqueous layer was extracted with $CHCl_3$ (2 × 100 ml). The combined chloroform extracts were washed with water, dried, and distilled. The yield of I was 11.8 g (16.0%), bp 74° C (35 mm). Found, %: C 63.41; H 6.03; N 12.60. Calculated for $C_5H_7NO_2$, %: C 63.09; H 6.22; N 12.37.

The diene synthesis of the 5-ethoxyoxazoles with maleic esters was performed by heating 0.1 mole of an oxazole with 0.2 mole of dimethyl or diethyl maleate at 110° C for 2 hr. Then the reaction mixture was cooled and was treated with 20 ml of a 25% solution of HCl in absolute methanol (or ethanol). The product was isolated by one of the following methods: a) the crystals that deposited were filtered off and were washed with a small amount of methanol and then with ether; or b) the reaction mixture was cooled, carefully mixed with 300 ml of water, and left overnight in the refrigerator. The crystals were filtered off and washed with ether. The compounds obtained are given in Table 2.

With cooling and stirring, potassium carbonate was added to a saturated aqueous solution of the hydrochloride to give a pH of 6.5-7. The mixture was carefully extracted with chloroform, the extract was dried, and the chloroform was distilled off in vacuum. The free bases obtained are given in Table 2.

Reduction of the diesters of 3-hydroxycinchomeronic acids. With cooling and stirring, a solution of 10 mM of one of the diesters in 50 ml of absolute ether (or tetrahydrofuran) was added dropwise to a suspension of 1.14 g (30 mM) of lithium aluminum hydride in 50 ml of absolute ether (or tetrahydrofuran). The mixture was kept at a gentle boil for 6 hr and was left overnight at room temperature. With cooling and stirring, 100 ml of water was added to the mixture and it was saturated with carbon dioxide. The precipitate was filtered off mixed with 100 ml of a mixture of water and ethanol (1:1) and again saturated with carbon dioxide. After filtration, the residue was washed with hot ethanol (2 × 50 ml), and the combined extracts were evaporated to dryness in vacuum at 45-50° C. The residue was extracted with hot ethanol (5 × 20 ml). The further isolation was carried out by one of the following methods: a) the combined extracts were evaporated to dryness in vacuum and the free base was washed with a small amount of cold acetone; or b) the combined extracts were evaporated in vacuum to small volume and then 2 ml of a 25% solution of HCl in absolute ethanol was added, followed by absolute ether until crystals began to deposit. The solution was left overnight in the refrigerator and the crystals of the hydrochloride that had deposited were filtered off and washed with ether. The compounds obtained are given in Table 3.

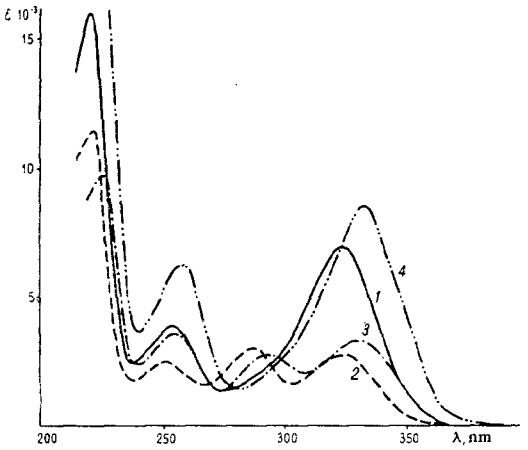


Fig. 1. UV spectra of pyridoxine and analogs in a phosphate buffer, pH 6.9: 1) pyridoxine; 2) 2-norpyridoxine; 3) 6-methyl-2-norpyridoxine; 4) 6-methylpyridoxine.

Table 1

Correlation of the Ionic Forms in the UV Spectra of Pyridoxal and Its Analogs

Ionic forms	Medium	λ_{max}, nm (ε · 10⁻³)			
		$R'=CH_3$*; $R''=H$	$R'=R''=H$	$R'=H$; $R''=CH_3$	$R'=R''=CH_3$
Pyridoxines					
The cation XI	0.1 *N* HCl	291 (8.6)	289 (6.4)	297 (6.2)	298 (10.3)
The bipolar ion XII	pH 6.9	254 (3.9)	251 (2.5)	256 (3.6)	257 (6.3)
		324 (7.2)	324 (2.8)	331 (3.3)	332 (8.4)
The neutral form XIII	pH 6.9	286 (5.7)**	286 (3.0)	294 (2.7)	—
The anion XIV	0.1 *N* KOH	245 (6.3)	242 (7.2)	247 (7.2)	248 (8.5)
		310 (6.8)	310 (5.6)	317 (5.2)	317 (7.9)
Pyridoxals					
The cation XV	0.1 *N* HCl	288 (9.0)	284 (6.6)	292 (6.4)	295 (8.3)
The bipolar ion XVI	pH 6.9	252 (5.8)	249 (4.6)	247 (4.7)	250 (5.3)
		317 (8.9)	314 (4.2)	323 (4.0)	324 (7.3)
The neutral form XVII	pH 6.9	280 (4.1)***	280 (2.1)	290 (2.1)	—
The anion XVIII	0.1 *N* KOH	240 (8.4)	240 (8.6)	240 (9.0)	242 (8.4)
		302 (5.7)	300 (5.0)	307 (5.6)	310 (7.8)
The anion XIX	0.1 *N* KOH	390 (1.7)	390 (0.6)	—	—
Pyridoxamines					
The dication XX	0.1 *N* HCl	292 (8.2)	292 (6.8)	299 (7.4)	302 (8.8)
The tripolar ion XXI	pH 6.9	252 (4.5)	251 (2.6)	253 (4.8)	255 (6.3)
		326 (7.9)	324 (3.2)	328 (4.1)	333 (8.7)
The monocation XXII	pH 6.9	287 (3.4)****	287 (3.0)	297 (2.9)	—
The anion XXIII	0.1 *N* KOH	245 (6.2)	243 (7.5)	245 (7.8)	248 (7.2)
		310 (7.2)	307 (5.9)	312 (5.8)	314 (7.2)

*The spectra of pyridoxine, pyridoxal, and pyridoxamine are given for comparison [8].

**Ethanol

***In 60% dioxane

****In 98% dioxane

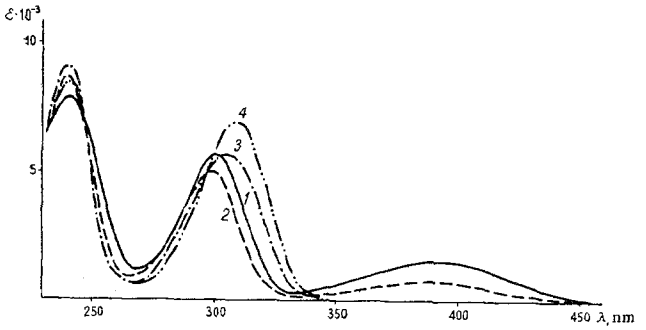


Fig. 2. UV spectra of pyridoxal and its analogs in 0.1 N KOH. Symbols as for Fig. 1.

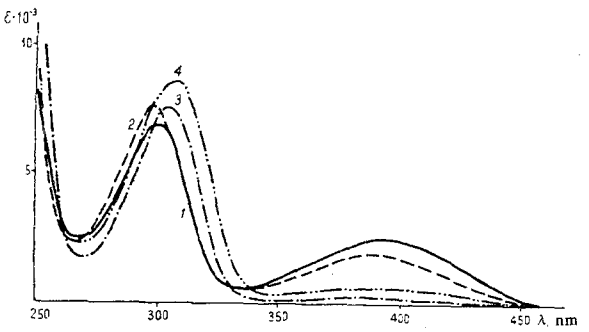


Fig. 3. UV spectra of the Schiff's bases with p-phenetidine in 0.1 N KOH. Symbols as for Fig. 1.

Table 2

Diene synthesis of 5-ethoxyoxazoles

Oxazole	Maleic diester	Compound obtained	Mp of the hydrochloride, °C	Yield of the hydrochloride, %	Method of isolation	Mp of the base, °C	Empirical formula	Found, %			Calculated, %			λ_{max}, nm ($\varepsilon \cdot 10^{-3}$) in 0.1 N KOH
								C	H	N	C	H	N	
5-Ethoxyoxazole	Dimethyl maleate	Dimethyl 3-hydroxycinchomeronate*	200—201	43—44	a	136—137								251 (5.8) 327 (5.4)
5-Ethoxy-2-methyloxazole	Dimethyl maleate	Dimethyl 3-hydroxy-6-methylcinchomeronate	123—124	28—30	b	57—58	$C_{10}H_{12}ClNO_6$	45.63	4.38	5.61	45.90	4.62	5.35	252 (5.9)*** 338 (5.2)
5-Ethoxy-2-methyloxazole	Diethyl maleate	Diethyl 3-hydroxy-6-methylcinchomeronate	153—154	19—21	b	Oil	$C_{12}H_{16}ClNO_5$	49.32	5.72	5.06	49.74	5.57	4.83	255 (5.4)*** 341 (5.4)
5-Ethoxy-2,4-dimethyloxazole	Dimethyl maleate	Dimethyl 2,6-dimethyl-3-hydroxycinchomeronate	166—167	41—43	b	56—57	$C_{11}H_{14}ClNO_5$	47.66	4.98	5.32	47.92	5.12	5.08	260 (4.3)*** 341 (6.5)
5-Ethoxy-2,4-dimethyloxazole	Diethyl maleate	Diethyl 3-hydroxy-2,6-dimethylcinchomeronate**	144—145	34—36	b	Oil	$C_{13}H_{18}ClNO_5$	51.06	5.62	4.94	51.40	5.97	4.61	262 (4.2) 345 (6.4)

*According to the literature [11], mp of the hydrochloride 195–197° C, mp of the base 129–133° C.

**According to the literature [12], mp of the hydrochloride 145–147° C.

***Inflection on the curve.

Table 3
Synthesis of Pyridoxine Analogs

Compound	Solvent	Method of isolation	Mp, °C	Empirical formula	Found, %			Calculated, %			Yield, %
					C	H	N	C	H	N	
2-Norpyridoxine*	Tetrahydrofuran	a	125—126 (decomp.)	—	—	—	—	—	—	—	71
6-Methyl-2-norpyridoxine**	Ether	b	197—199 (decomp.)	$C_8H_{11}NO_3$	56.49	6.81	8.13	56.77	6.58	8.26	56—61
6-Methylpyridoxine ***	Ether	b	174—179 (decomp.)	$C_9H_{13}NO_3$	59.02	7.40	7.51	58.99	7.16	7.65	60—64

*According to the literature [11], mp 124-125° C.

**Mp of the hydrochloride 173-175° C.

***Mp of the hydrochloride 146-148° C.

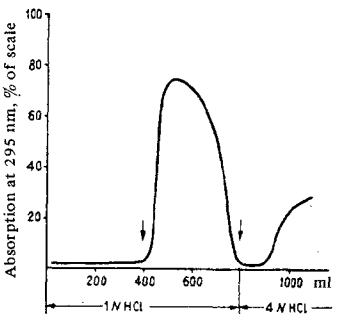


Fig. 4. Isolation of 6-methylpyridoxal in the chromatography of 6-methyl-pyridoxylidene-p-phenetidine on Dowex 50W × 4 in the acid form (absorption at 295 nm).

Table 4
Derivatives of Analogs of Pyridoxine

Compound	Mp, °C	λ_{max}, nm ($\varepsilon \times 10^{-3}$) in 0.1 N KOH	Empirical formula	Found, %			Calculated, %			Yield, %
				C	H	N	C	H	N	
2-Norpyridoxal oxime	201—203 (decomp.)	243 (12.4) 278 (7.3) 350 (9.4)	$C_7H_8N_2O_3$	49.76	4.90	16.43	50.0	4.80	16.66	75
6-Methyl-2-norpyridoxal oxime	185—187 (decomp.)	243 (12.6) 280* (6.9) 352 (7.1)	$C_8H_{10}N_2O_3$	52.62	5.67	15.20	52.74	5.53	15.38	59
6-Methylpyridoxal oxime	209—212 (decomp.)	243 (12.9) 280* (6.5) 354 (7.3)	$C_9H_{12}N_2O_3$	54.78	6.29	14.31	55.09	6.17	14.28	69
2-Norpyridoxylidene-p-phenetidene	192—194 (decomp.)	235 (19.5) 298 (7.7) 390 (1.9)	$C_{15}H_{16}N_2O_3$	66.50	5.61	10.47	66.14	5.92	10.27	79
6-Methyl-2-norpyridoxylidene-p-phenetidine	179—183 (decomp.)	238 (20.5) 304 (7.5) 390 (0.2)	$C_{16}H_{18}N_2O_3$	67.35	6.51	9.59	67.13	6.33	9.88	38
6-Methylpyridoxylidene-p-phenetidine	177—180 (decomp.)	238 (16.5) 308 (9.7) 390 (0.8)	$C_{17}H_{20}N_2O_3$	67.76	6.83	9.02	68.00	6.70	9.33	66

*Inflection on the curve.

Table 5
Analogs of Pyridoxal and Pyridoxamine

Compound	Mp, °C	Empirical formula	Found, %			Calculated, %			Yield, %
			C	H	N	C	H	N	
2-Norpyridoxal hydrochloride	144—147 (decomp.)	$C_7H_7NO_3 \cdot HCl$	44.15	4.17	7.51	44.35	4.25	7.38	93—95
6-Methyl-2-norpyridoxal hydrochloride	Decomp. above 170	$C_8H_9NO_3 \cdot HCl$	47.04	5.12	6.63	47.20	4.95	6.88	94—95
6-Methylpyridoxal hydrochloride	Decomp. above 170	$C_9H_{13}NO_3 \cdot HCl$	49.39	5.67	6.19	49.66	5.56	6.44	92—94
2-Norpyridoxamine dihydrochloride	165—170 (decomp.)	$C_7H_{10}N_2O_2 \cdot 2HCl$	37.26	5.25	12.40	37.02	5.33	12.34	96—98
6-Methyl-2-norpyridoxamine dihydrochloride	234—240 (decomp.)	$C_8H_{12}N_2O_2 \cdot 2HCl$	39.61	5.77	11.93	39.86	5.85	11.62	96—98
6-Methylpyridoxamine dihydrochloride	181—184 (decomp.)	$C_9H_{14}N_2O_2 \cdot 2HCl$	42.12	6.61	10.69	42.37	6.32	10.98	96—98

Oxidation of the pyridoxine analogs with manganese dioxide. To a solution of 3 mM of the diol in 0.3 M H_2SO_4 (in the oxidation of the free base, in 15 ml; in the oxidation of the hydrochloride, in 10 ml) was added 270 mg of manganese dioxide "B" [10]. The mixture was stirred at room temperature with a magnetic stirrer until the manganese dioxide had dissolved completely (3 hr).

Isolation in the form of the oxime. Then, 310 mg of hydroxylamine hydrochloride was added to the reaction mixture and it was heated to 70° C and 820 mg of anhydrous sodium acetate was added. Heating at the same temperature was continued for another 10 min and then the mixture was left in the refrigerator for 2 hr. The crystals of the oxime were filtered off and washed with ice water.

Isolation in the form of the Schiff's base. Finally, 8 ml of a 0.5 M solution of p-phenetidine hydrochloride and then 12 ml of a 2 N solution of sodium acetate were added to the mixture. It was left in the refrigerator for 2 hr, and the precipitate was filtered off and carefully washed with ice water. The compounds obtained are given in Table 4.

Hydrolysis of the Schiff's bases. One of the Schiff's bases with p-phenetidine (300 mg) was dissolved in 5 ml of 1 N HCl and deposited on a 1.4 × 35 cm column of Dowex 50W × 4 in the acid form equilibrated with 1 N HCl. Elution was carried out with 1 N HCl at the rate of 40 ml per hour. The fractions containing the aldehyde (from the absorption at 295 nm) were evaporated to dryness in vacuum at 40–45° C. The separation curve is given in Fig. 4. The substances obtained are given in Table 5.

Hydrogenation of the oximes. 150 mg of 5% Pd/C was added to a solution of 1 mM of an oxime in 20 ml of water and 0.5 ml of conc HCl, and hydrogenation was carried out at room temperature. The theoretical volume of hydrogen was absorbed after 30–45 min. The catalyst was filtered off and washed with hot water, and the combined filtrates were evaporated to dryness in vacuum. The compounds obtained are given in Table 5.

All the substances obtained were homogeneous on chromatography in a super thin layer in the ethyl acetate–acetone–25% ammonia (20 : 10 : 1.5) system.

The UV spectra were taken on an SF-4A instrument at a layer thickness of 1 cm with concentrations of 10^{-4} M.

REFERENCES

1. A. E. Braunshtein and M. M. Shemyakin, Biokhimiya, **18**, 393, 1958.

2. D. E. Metzler, M. Ikawa, and E. E. Snell, J. Am. Chem. Soc., **76**, 648, 1954.

3. G. Ya. Kondrat'eva and Huang Chih-Heng, DAN, **141**, 628, 861, 1961.

4. E. E. Harris, R. A. Firestone, K. Pfister, R. R. Boettcher, F. J. Cross, R. B. Currie, M. Monaco, E. R. Peterson, and W. Reuter, J. Org. Chem., **27**, 2705, 1962.

5. P. Karrer and Ch. Gränacher, Helv. Chim. Acta, **7**, 763, 1924.

6. P. Karrer, E. Miyamichi, H. C. Storm, and R. Widmer, Helv. Chim. Acta, **8**, 205, 1925.

7. R. A. Firestone, E. E. Harris, and W. Reuter, Tetrah., **23**, 943, 1967.

8. D. E. Metzler and E. E. Snell, J. Am. Soc., **77**, 243, 1955.

9. H. Wada and E. E. Snell, J. Biol. Chem., **236**, 2089, 1961.

10. M. Harnfest, A. Bavely, and W. A. Lazier, J. Org. Chem., **19**, 1608, 1954.

11. S. M. Gadekar, I. L. Frederik, and E. S. de Renzo, J. Med. Pharm. Chem., **5**, 531, 1962.

12. Takeo Naito and Toru Yoshikawa, Chem. Pharm. Bull., Japan, **14**, 918, 1966.

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