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30. Masao Uchibayashi : New Fat-soluble Derivatives of Pyridoxine prepared by Selective Acylation.

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Sakuragi and Kummerow reported the synthesis of several pyridoxine triacylates[1] and showed that these pyridoxine derivatives were soluble in fat and stable toward heat, and retained the full pyridoxine activity.[2] In an attempt to prepare pyridoxine tripalmitate, their procedure was repeated in this laboratory by treating pyridoxine hydrochloride (I) with three equivalents of palmitoyl chloride in a mixture of chloroform and pyridine. The product was unexpectedly found to be pyridoxine dipalmitate, m.p. 88~89°, judged by the analytical and infrared spectral data. Since the compound was not identical with the known pyridoxine 3,5-dipalmitate,[1] m.p. 58~61°, in melting point and ultraviolet spectrum, and gave a negative ferric chloride test, it was presumed to be the previously undescribed pyridoxine 3,4-dipalmitate (IIb). The tripalmitate (IIIb), the initially desired compound, was obtained by using pyridine as a sole solvent in the above acylation.

As to the study of fatty acid derivatives of pyridoxine, reports on the acetate[3~6] and propionate[5] have been published besides the ones mentioned above,[1,2] but no mention has been made on such a selective acylation as was encountered in the present investigation. Assuming that the selectivity might be related to the molecular size of fatty acid used, acylation was examined with a series of fatty acids. The results obtained showed that the selective esterification was common to all the acids and, as was expected, yield of the diacylates was poor in the case of short-chain fatty acids and excellent when long-chain acids were employed. The acylation, however, was found to be attended by the formation of a small amount of the corresponding triacylate. Of these concomitant products the compounds esterified by fatty acids shorter than octanoic acid appear to be liquid at room temperature and no attempt was made to isolate them from the mother liquor of the respective diacylate. Eleven pyridoxine diacylates (II) and three pyridoxine triacylates (III) synthesized in the present work (Table I) are all new fat-soluble derivatives of pyridoxine.

Structure of the diacylates was then investigated. Reaction of pyridoxine hydrochloride (I) with 3.1 moles of acetyl chloride in a mixture of chloroform and pyridine at room temperature afforded a compound (IIa), m.p. 162°, which had an elemental composition corresponding to pyridoxine diacetate hydrochloride and gave a negative ferric chloride test. Of the three possible isomers of pyridoxine diacetate, the 4,5-diacetate (IX) has already been recorded. Thus, the compound (IX) was obtained by refluxing pyridoxine with hydrobromic acid,[7] followed by treatment of the resultant dibromide (VIII) with silver acetate.[8] Mixed melting point, infrared spectra, and other properties confirmed that the compound (IIa) in question was not identical with the 4,5-diacetate (IX).

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1) T. Sakuragi, F. A. Kummerow : J. Am. Chem. Soc., **78**, 839 (1956).
2) *Idem* : J. Am. Oil Chemists' Soc., **33**, 116 (1956); J. Nutrition, **58**, 557 (1956); Arch. Biochem. Biophys., **63**, 32 (1956); T. Sakuragi : J. Org. Chem., **23**, 129 (1958).
3) A. Ichiba, K. Michi : Sci. Papers Inst. Phys. Chem. Research (Tokyo), **35**, 73 (1938); R. Kuhn, G. Wendt : Chem. Ber., **71**, 780 (1938).
4) S. A. Harris : J. Am. Chem. Soc., **62**, 3203 (1940).
5) R. Kuhn, G. Wendt : U. S. Pat. 2,296,167 (C. A., **37**, 1229 (1943)); S. A. Harris, E. T. Stiller : U. S. Pat. 2,349,267 (C. A., **39**, 1514 (1945)); J. C. Keresztesy, J. R. Stevens : U. S. Pat. 2,412,272 (C. A., **41**, 2212 (1947)).
6) S. A. Harris, D. Heyl, K. Folkers : J. Am. Chem. Soc., **66**, 2088 (1944).
7) S. A. Harris, K. Folkers : *Ibid.*, **61**, 1245 (1939).
8) S. A. Harris : *Ibid.*, **62**, 3203 (1940).

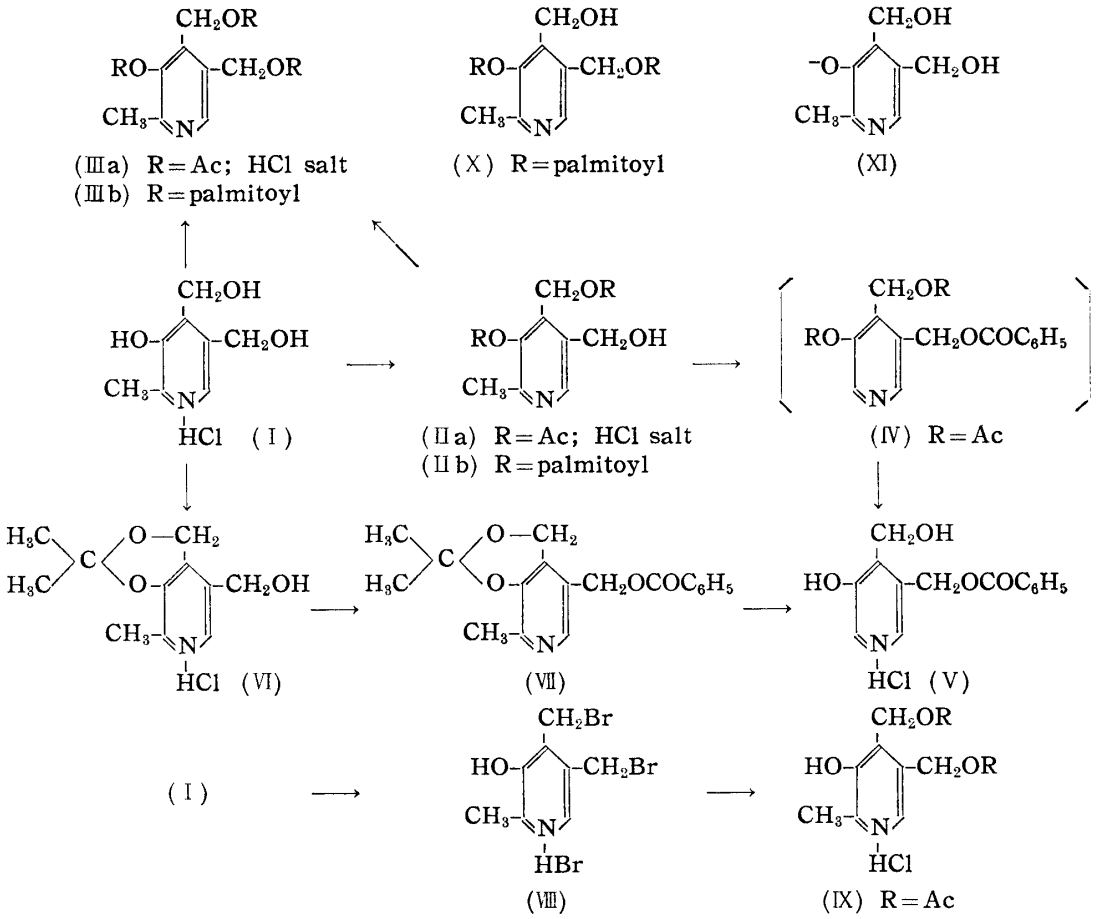

Table I. Pyridoxine Diacylates and Triacylates

Pyridoxine acylate	m.p. (°C)	Yield (%)	UV[g] λmax (mμ)	UV[g] ε (×10⁻³)	Mol. formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
Diacetate·HCl[b]	162	16.5	274	4.52	$C_{12}H_{16}O_5NCl$	49.75	5.56	4.83	50.03	5.83	4.73
Dipropionate[c]	83~84	25.0	272	4.39	$C_{14}H_{19}O_5N$	59.77	6.81	4.98	59.99	7.03	4.93
Dibutyrate[d]	56~58	10.0	273	4.38	$C_{16}H_{23}O_5N$	62.12	7.49	4.53	62.27	7.69	4.58
Divalerate[e]	65~67	51.5	273	4.31	$C_{18}H_{27}O_5N$	64.07	8.06	4.15	64.07	7.88	4.10
Dihexanoate[d]	66~67	21.3	273	4.51	$C_{20}H_{31}O_5N$	65.73	8.55	3.83	65.70	8.85	3.93
Dioctanoate[e]	69~71	28.6	272	4.49	$C_{24}H_{39}O_5N$	68.37	9.33	3.32	68.49	9.24	3.35
Didecanoate[b]	73~74	44.2	272	3.91	$C_{28}H_{47}O_5N$	70.40	9.92	2.93	70.63	10.07	2.77
Dilaurate[b]	79~80	62.0	272	4.49	$C_{32}H_{55}O_5N$	72.00	10.38	2.62	71.90	10.42	2.40
Dimyristate[f]	85~86	70.0	272	3.98	$C_{36}H_{63}O_5N$	73.30	10.77	2.37	73.61	10.86	2.20
Dipalmitate[f]	88~89	75.5	271	3.90	$C_{40}H_{71}O_5N$	74.37	11.08	2.17	74.55	10.82	2.07
Distearate[f]	90~92	77.0	272	4.25	$C_{44}H_{79}O_5N$	75.30	11.34	1.99	75.56	11.48	2.10
Tridecanoate[a,b]	47~48	—	272	4.68	$C_{38}H_{65}O_6N$	72.22	10.37	2.22	72.11	10.23	2.26
Trilaurate[b]	55~56	—	272	4.50	$C_{44}H_{77}O_6N$	73.80	10.84	1.96	74.12	10.93	2.02
Trimyristate[f]	66~68	—	272	4.40	$C_{50}H_{89}O_6N$	75.04	11.21	1.75	74.87	10.95	1.98
Tripalmitate[a,f]	72~74	—	271	4.40	$C_{56}H_{101}O_6N$	76.04	11.51	1.58	75.72	11.54	1.48
Tristearate[f]	78~79	—	271	4.32	$C_{62}H_{113}O_6N$	76.88	11.76	1.44	76.89	11.55	1.52

a) The compound described previously.
b) Recrystallized from EtOH.
c) Recrystallized from a mixture of Me_2CO and hexane.
d) Recrystallized from ligroine.
e) Recrystallized from hexane.
f) Recrystallized from 2-propanol.
g) The spectra of the di- and trimyristates, di- and tripalmitates, and di- and tristearates were measured in Et_2O and those of other compounds in EtOH.

Treatment of compound (IIa) with acetic anhydride and pyridine provided pyridoxine triacetate (IIIa) which was proved to be identical with the triacetate prepared by acetylation of pyridoxine in the usual manner.[8] The latter triacetylation was found to be accompanied by the formation of compound (IIa) when the reaction was conducted under milder conditions. Compound (IIa) was further converted by the treatment with benzoyl chloride in pyridine into a diacetyl benzoate (IV), which was then subjected to mild hydrolysis to remove the acetyl groups. Pyridoxine benzoate (V) thus obtained gave a good ferric chloride reaction and must be either the 4- or 5-benzoate. To ascertain the structure of (V), pyridoxine 5-benzoate was prepared starting from isopropylidenepyridoxine hydrochloride (VI).[9,10] The choice of this starting material was suggested by the preparation of pyridoxine 5-palmitate reported by Sakuragi and Kummerow.[1]

The acetonide (VI) was prepared by treating pyridoxine hydrochloride (I) with acetone in the presence of sulfuric acid, as reported by Cohen and Hughes.[10] This reaction, however, did not appear to occur when chlorosulfonic acid was used as catalyst. Esterification of the hydroxyl group which remained unprotected in (VI) was reported to be unsuccessful with phosphoryl chloride or *p*-toluenesulfonyl chloride, but attainable with *p*-nitrobenzoyl chloride.[9] In the present experiment, benzoylation of (VI) with benzoyl chloride proceeded smoothly in pyridine and isopropylidenepyridoxine 5-benzoate (VII) was obtained. It is generally known that an isopropylidene moiety of acetonides is easily cleaved by acid treatment. In the present case, refluxing of compound (VII) in 80% acetic acid did not cause hydrolysis and the treatment of (VII) with dilute hydrochloric acid in ethanol resulted in the formation of desired pyridoxine 5-benzoate (V). The product (V) was proved to be identical with the benzoate derived from the diacetate (IIa), by comparison of their infrared spectra and mixed melting point determination. The diacetate (IIa) obtained by selective esterification was thus verified to be pyridoxine 3,4-diacetate which was not recorded so far in the literature.

Other diacylates (II) may safely be regarded as 3,4-diacylates by analogy with the diacetate (IIa). Their structure is further supported by the following consideration. 3-Hydroxypyridine derivatives are known to exhibit no absorption band corresponding to the hydroxyl group in the 3-μ region of the infrared spectrum, due probably to an intermolecular hydrogen bonding.[11] The presence of 3-hydroxyl in those compounds, therefore, can hardly be predicted by means of the spectrum, but is detectable by the ferric chloride test. Every diacylate obtained in the present study possesses a distinct absorption at around 3 μ in its spectrum, indicating that the hydroxyl group of either the 4- or 5-hydroxymethyl group remains free and the compound gives a negative ferric chloride test. These findings show that it may be either the 3,4- or 3,5-diacylate. Further, the possibility of the latter can be ruled out when consideration is given to the structure of the diacetate (IIa) and to the fact that the dipalmitate (IIb) is not identical with the known 3,5-dipalmitate (X). It may thus be concluded that the diacyl derivatives (II) of pyridoxine are all 3,4-diacylates.

The resuts of the present experiment disclosed that on mild acylation of pyridoxine the 3-hydroxyl group and the hydroxyl of the 4-hydroxymethyl group were preferably attacked. Since pyridoxine is believed to take the form of (XI) in basic media,[12] the acylation at the 3-hydroxyl may be easier.[14] The hydroxyl group of the 4-hydroxymethyl may also be reactive because of its *para*-location to the nitrogen in the pyridine ring.[13] Such

9) J. Baddiley, A. P. Mathias : J. Chem. Soc., **1952**, 2583.
10) A. Cohen, E. G. Hughes : *Ibid.*, **1952**, 4384.
11) D. Heinert, A. E. Martell : J. Am. Chem. Soc., **81**, 3933 (1959).
12) R. C. Elderfield : "Heterocyclic Compounds," **1**, 606 (1950). John Wiley & Sons, Inc., New York.
13) *Idem* : *Ibid.*, **1**, 408 (1950).
14) A. R. Katritzky, J. M. Lagowaki : "Heterocyclic Chemistry," 89 (1960). Methuen & Co. Ltd., London.

difference in reactivity of the hydroxyl groups may offer one of the possible interpretations for the selective esterification.

Biological test*[2] of pyridoxine 3,4-dipalmitate (IIb) for its inhibitory effect on OMP*[3]-induced convulsion in mice showed that the compound was as active as pyridoxine on a molar basis when administered orally or together with the diet. The diacyl pyridoxines esterified by long-chain fatty acids show good promise for wide application in pharmaceutical preparations as stable, fat-soluble derivatives of pyridoxine.

Experimental*[4]

Diacetylation of Pyridoxine—a) General procedure for 2-methyl-3-acetoxy-4-acetoxymethyl-5-hydroxymethylpyridine hydrochloride (pyridoxine 3,4-diacetate hydrochloride) (IIa): To a suspension of 6.5 g. of pyridoxine hydrochloride (I) in a mixture of 160 cc. of $CHCl_3$ and 170 cc. of pyridine, 7.7 g. of AcCl dissolved in 7 cc. of $CHCl_3$ was added dropwise with stirring at room temperature. After completion of the addition, the mixture was stirred for 4 hr. The resultant solution was mixed with 200 cc. of $CHCl_3$, washed with H_2O, and dried over anhyd. $MgSO_4$. Evaporation of the solvent gave an oily residue which was treated with HCl in Et_2O. The precipitate was recrystallized from EtOH to 4.8 g. of colorless fine needles, m.p. 162°. The product showed a negative $FeCl_3$ reaction.

b) General procedure for pyridoxine dipropionate, dibutyrate, divalerate, dihexanoate, and dioctanoate: To a suspension of 2.05 g. of (I) in a mixture of 40 cc. of $CHCl_3$ and 50 cc. of pyridine, 0.03 mole of the fatty acid chloride dissolved in 10 cc. of $CHCl_3$ was added with stirring at room temperature over a period of 40 min. After being stirred for further 4 hr., the mixture was allowed to stand overnight, mixed with 200 cc. of $CHCl_3$, washed with H_2O, and dried over anhyd. $MgSO_4$. Evaporation of the solvent gave a syrup which partially crystallized on standing in the cold. The crystals were separated by filtration and purified by recrystallization from a suitable solvent. The products exhibited an absorption of a hydroxyl group in the infrared spectra and gave a negative $FeCl_3$ test.

c) General procedure for pyridoxine didecanoate, dilaurate, dimyristate, dipalmitate, and distearate: In this case, the corresponding triacylate was isolated as a by-product from each compound.

The reaction was effected in the same manner as procedure (b) and the mixture was set aside at room temperature for 40~60 hr. The resultant solution was mixed with 200 cc. of $CHCl_3$, washed successively with H_2O, dil. HCl, dil. Na_2CO_3 solution, and H_2O. After being dried over anhyd. $MgSO_4$, the solvent was evaporated to dryness and the residue was subjected to fractional recrystallization from a suitable solvent. A small amount of the triacylate precipitated and the desired compound was obtained from the mother liquor. The diacylates prepared by this procedure showed a band corresponding to a hydroxyl in the infrared spectra, and gave a negative $FeCl_3$ test.

2-Methyl-3-acetoxy-4,5-diacetoxymethylpyridine Hydrochloride (Pyridoxine Triacetate Hydrochloride) (IIIa)—(i) From pyridoxine hydrochloride (I): A mixture of 2 g. of (I), 20 cc. of pyridine, and 20 cc. of Ac_2O was treated as described in the literature[8] and 2.44 g. of colorless needles, m.p. 155~158° (reported[8] m.p. 157°), was obtained. From the mother liquor, 0.30 g. of pyridoxine 3,4-diacetate hydrochloride (IIa), m.p. 159~161.5°, was isolated and characterized by infrared spectrum.

(ii) From pyridoxine 3,4-diacetate hydrochloride (IIa): A solution of 124 mg. of (IIa) in a mixture of 5 cc. of pyridine and 5 cc. of Ac_2O was allowed to stand overnight and then heated at 80° for 30 min. The mixture was concentrated to dryness *in vacuo* and the residue was dissolved in Et_2O, which was washed with H_2O and dried over anhyd. $MgSO_4$. Introduction of HCl to this Et_2O solution caused immediate precipitation. Recrystallization from EtOH yielded colorless needles, m.p. 155~157.5°. It gave a negative $FeCl_3$ test. *Anal.* Calcd. for $C_{14}H_{18}O_6NCl$: C, 50.68; H, 5.47; N, 4.22. Found: C, 50.60; H, 5.53; N, 4.32.

2-Methyl-3-palmitoxy-4,5-dipalmitoxymethylpyridne (Pyridoxine Tripalmitate) (IIIb)—To a suspension of 1.2 g. of (I) in 37 cc. of pyridine, 5.3 g. of palmitoyl chloride was added with stirring at room temperature during 30 min. The resultant semi-solid was mixed with 10 cc. of pyridine and allowed to stand for 30 hr. at room temperature. The mixture was extracted with $CHCl_3$ and the extract was washed successively with H_2O, dil. HCl, and H_2O, and dried over anhyd. Na_2SO_4. Evaporation of the $CHCl_3$ solution and crystallization of the residue from 2-propanol afforded 3.5 g. of an amorphous powder, m.p. 72~74° (reported m.p. 72~74°).

*[2] Conducted and to be published in detail by Dr. S. Shintani of the Test Division, to whom the author is grateful.

*[3] OMP=2-methyl-4-amino-5-pyrimidinemethanol; see S. Shintani: Yakugaku Zasshi, **77**, 736 (1957).

*[4] All melting points are uncorrected and the infrared spectra were measured in Nujol mulls.

2-Methyl-3-hydroxy-4-hydroxymethyl-5-benzoyloxymethylpyridine Hydrochloride (Pyridoxine 5-Benzoate Hydrochloride) (V)—(i) From pyridoxine 3,4-diacetate hydrochloride (IIa): A solution of 800 mg. of (IIa) in a mixture of 540 mg. of BzCl and 5 cc. of pyridine was heated at 100° for 1 hr. The reaction mixture was poured into ice water and the oily layer was extracted with Et_2O. The Et_2O solution was washed with H_2O, dried over anhyd. $MgSO_4$, and then treated with HCl. The precipitate was dissolved in 80% EtOH and heated on a water bath for about 20 min. On evaporation *in vacuo* the solution gave an oily residue which was allowed to stand in the cold to yield 780 mg. of crystals. Recrystallization from EtOH gave colorless needles, m.p. 175~176.5°. *Anal.* Calcd. for $C_{15}H_{16}O_4NCl$: C, 58.17; H, 5.20; N, 4.55. Found: C, 58.24; H, 5.16; N, 4.50. The product gave a good $FeCl_3$ test and was confirmed by mixed melting point and infrared spectrum to be identical with the compound prepared by the following method.

(ii) From isopropylidenepyridoxine 5-benzoate (VII): A mixture of 500 mg. of (VII), 0.3 cc. of conc. HCl, 10 cc. of EtOH, and 5 cc. of H_2O was refluxed for 10 min. and evaporated *in vacuo*. The residue was recrystallized from EtOH to give 250 mg. of colorless needles, melting at 179°. *Anal.* Found: C, 58.18; H, 5.47; N, 4.45.

Isopropylidenepyridoxine Hydrochloride (VI)—As indicated in the literature,[10] the compound was perpared by the reaction of 12 g. of (I) with 240 cc. of Me_2CO containing 24 cc. of conc. H_2SO_4. Colorless needles, m.p. 212° (decomp.) (reported[10] m.p. 217~218°); yield, 8.65 g.

Isopropylidenepyridoxine 5-Benzoate (VII)—A solution of 1.0 g. of (VI) and 0.6 cc. of BzCl in 6 cc. of pyridine was heated at 100° for 1 hr. The reaction mixture was poured into ice water and extracted with Et_2O. The Et_2O extract was washed with H_2O, dried over anhyd. Na_2SO_4, and evaporated. Recrystallization from petr. benzine furnished 1.05 g. of colorless rhombic crystals, m.p. 85~86°. *Anal.* Calcd. for $C_{18}H_{19}O_4N$: C, 68.99; H, 6.11; N, 4.47. Found: C, 69.18; H, 6.02; N, 4.73.

2-Methyl-3-hydroxy-4,5-diacetoxymethylpyridine Hydrochloride (Pyridoxine 4,5-Diacetate Hydrochloride) (IX)—This compound was obtained by the methods reported previously.[7,8] A solution of 3 g. of (I) in 150 cc. of 40% HBr was refluxed for 30 min. and 1.9 g. of the dibromide (VIII) was obtained, m.p. 223~225° (decomp.), which exhibited no absorption of a hydroxyl group in its infrared spectrum. Then, a mixture of 1.9 g. of (VIII), 2.55 g. of AcOAg, and 11 g. of AcOK in 50 cc. of glacial AcOH was boiled for 1 hr. The product was crystallized from EtOH into colorless needles, m.p. 159~161°, which gave a positive $FeCl_3$ reaction and possessed no band indicative of a hydroxyl group in its infrared spectrum. UV: λ_{max}^{EtOH} 294 mμ (ε 7.28×10^3).

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Summary

Acylation of pyridoxine (I) with fatty acid chloride in pyridine and chloroform at room temperature gave pyridoxine diacylates (II), which were verified to be the 3,4-diacylates. The diacylates prepared and the triacylates obtained as a by-product are listed in Table I. The long-chain fatty acid derivatives (II) are stable to heat and soluble in fat. Pyridoxine 3,4-dipalmitate (IIb) showed a complete pyridoxine activity in mice by oral or dietary administration.

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