Furoyl and Furfuryl Derivatives of Pyridoxamine (Kinetin Analogs)

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Kinetin,[2] or 6-furfurylaminopurine (I), is a potent cell-division accelerator, first obtained as an artifact[3] from autoclaved deoxyribonucleic acid (DNA). A novel hypothesis by Berglas[4] suggests that such accelerators (in contrast to traditional inhibitors) might be of value for tumor control. Preliminary experiments[5] on the effect of kinetin on sarcoma cells have been reported. Skinner and Shive[6] have recently prepared kinetin analogs with the furfuryl group replaced by other groups. We wish now to report the preparation of kinetin analogs with the purine group replaced by a pyridoxamine (II) residue.

Since direct acylation of pyridoxamine with one mole of furoyl chloride failed to yield any pure product, the monofuroyl derivative (VI) was obtained by first preparation and then selective hydrolysis of the previously unreported trifuroyl derivative (V).

Isolation of the free monofuroyl base, VI, proved quite difficult because of its high solubility. However, when saturated aqueous picric acid was added to the neutralized hydrolysis solution, the picrate of VI was obtained in high yield. The picrate was surprisingly insoluble in nearly all solvents, and probably for this reason was very difficult to regenerate by the usual procedures.

However, excellent results were finally obtained by triturating the solid picrate with an anhydrous solution of hydrogen chloride in glacial acetic acid. (Such a solution is conveniently prepared by adding acetic anhydride to concentrated aqueous hydrochloric acid.)

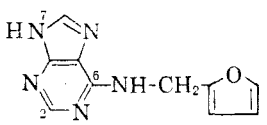
Under acidic conditions there is a tendency to N → O acyl migration[7] by acylaminoalkanols. On steric grounds, a migration to either the phenolic or the alcoholic oxygen should be possible in the case of the *N*-monofuroyl derivative, VI. These migrations would involve respectively, a six-membered and a seven-membered transient cyclic intermediate, each being forced into partial coplanarity by the pyridine ring.

The migration product, III or IV, might conceivably separate as its *mono*-hydrochloride (because of weak basicity of the ring nitrogen) which would be isomeric with the hydrochloride of VI. Thus the correct analysis which was obtained for VI (.HCl) does not rigidly exclude migration. However, other evidence eliminated the possibility of migration under the acidic conditions used: a test for primary amino (or ammonium) group was negative, and a test for phenolic hydroxyl was positive. Further, the infrared spectrum agreed with the non-migrated structure, VI (.HCl).

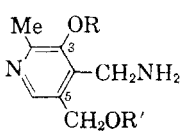
For the preparation of the secondary amine, VII, the reduction of the corresponding azomethine (Schiff's base)[8,9] (VIII) appeared most feasible. On hydrogenation, this azomethine rapidly reached and exceeded the theoretical (one mole) hydrogen uptake, and even with interruption at the one mole uptake level, no pure product could be isolated. Successful hydrogenations of other azomethines of pyridoxal have been reported.[9] Thus the difficulty is presumably due to gradual hydrogenation of the furan (not the pyridine) ring. However, by treatment of the azomethine with sodium borohydride,[10] the desired secondary amine (VII) was obtained.

The compounds V, VI (.HCl), VII, and VIII are being tested as possible anti-cancer agents in

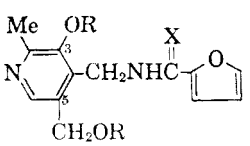
CHART I



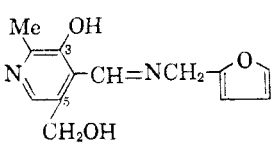
I



II, R = R′ = H
III, R = H, R′ = C_4H_3OCO
IV, R = C_4H_3OCO, R′ = H



V, R = C_4H_3OCO–, X = O
VI, R = H, X = O
VII, R = H, X = H, H



VIII

(1) For previous work by one of us on (pyrimidine) analogs of pyridoxine, see G. E. McCasland *et al.*, *J. Am. Chem. Soc.*, **74**, 842 (1952), and references there cited.

(2) C. O. Miller *et al.* (a) *J. Am. Chem. Soc.*, **77**, 2662 (1955); (b) **77**, 1392 (1955); (c) **78**, 1375 (1956).

(3) R. H. Hall and R. S. DeRopp, *J. Am. Chem. Soc.*, **77**, 6400 (1955).

(4) A. Berglas, *Cancer: Nature, Cause and Cure*, The Pasteur Institute, Paris, 1957, p. 84.

(5) Y. Ogawa *et al.*, *Nature*, **180**, 985 (1957).

(6) C. G. Skinner and W. Shive, *J. Am. Chem. Soc.*, **77**, 6692 (1955).

(7) G. E. McCasland, *J. Am. Chem. Soc.*, **73**, 2295 (1951)

(8) (a) Azomethines of pyridoxal resulting from interaction with amines, amino acids, peptides, or proteins have received considerable attention; (b) E. E. Snell *et al.*, *J. Am. Chem. Soc.*, **79**, 485 (1957); (c) *J. Am. Chem. Soc.*, **76**, 639 (1954); (d) *J. Am. Chem. Soc.*, **70**, 3432 (1948); (e) H. N. Christensen, *J. Am. Chem. Soc.*, **79**, 4073 (1957); (f) *J. Am. Chem. Soc.*, **80**, 99 (1958).

(9) A group at the Merck Research Laboratories has described the preparation of numerous azomethines of pyridoxal, and their platinum-catalyzed hydrogenation to secondary amines. Apparently no furan derivatives were included. See: (a) U. S. Patent **2,695,297**; *Chem. Abstr.*, **49**, 13297 (1955); (b) D. Heyl *et al.*, *J. Am. Chem. Soc.*, **70**, 1670, 3429, 3669 (1948).

(10) The reduction of various azomethines by sodium borohydride was described by J. H. Billman and A. C. Diesing, *J. Org. Chem.*, **22**, 1068 (1957).

these laboratories. Samples have also been submitted to Dr. Eugene Roberts of the City of Hope Medical Center, Duarte, Calif., for studies on the formation and metabolism of 4-aminobutyric acid. Results of these tests will be reported elsewhere.

EXPERIMENTAL

All melting and boiling points have been corrected. Melting points unless otherwise noted were determined with a *Monoscop* micro hot-stage. Microanalyses by Drs. Weiler and Strauss, Oxford, England, and by the Berkeley Analytical Laboratories, Berkeley, Calif.

2-Methyl-3-furoyloxy-4-furoylaminomethyl-5-furoyloxymethylpyridine (trifuroyl pyridoxamine). To a suspension of 964 mg. of commercial pyridoxamine dihydrochloride in 10 ml. of anhydrous pyridine was added all at once with magnetic stirring 1.58 ml. (2.08 gm.) of furoyl chloride. The nearly clear solution soon yielded a new precipitate. After a few minutes the entire mixture was added rapidly to a well-stirred solution of 1.6 g. of anhydrous sodium carbonate in 20 ml. of water. More water (20 ml.) was added, causing separation of an oil which changed into colorless needles after 20 min. continued stirring. The crystals were collected and washed (water: 20 ml., 20 ml.) giving 1.3 g. (dry weight) of snow white crude product, m.p. 85–90°.

This material dissolved readily in cold absolute ethanol (4 ml.) but within 2 min. new crystals separated[11] which required 6.5 ml. of boiling ethanol for solution. After adding another 2.5 ml. portion of ethanol, and cooling, colorless needles were obtained, dry weight 1.0 g. (56%), m.p. 131–132°.

Anal. Calcd. for $C_{23}H_{18}N_2O_8$: C, 61.32; H, 4.03; N, 6.22. Found: C, 61.66; H, 4.40; N, 6.12.

2-Methyl-3-hydroxy-4-furoylaminomethyl-5-hydroxymethylpyridinium picrate (4-N-furoyl pyridoxamine picrate). The trifuroyl derivative (450 mg.) was stirred for 2 hr. with 2.0 ml. ethanol and 4.0 ml. of *M* aqueous sodium hydroxide. To the resulting clear solution after adjustment to *p*H 5–7 (acetic acid) was added with stirring 229 mg. of picric acid in 18 ml. of water, giving a yellow precipitate, dry weight 490 mg. (nearly 100%), m.p. 215–220° (dec.).

This material was stirred with 50 ml. of boiling dioxane. The hot-filtered solution (residue 60 mg.) on cooling deposited yellow needles, dry weight 216 mg., m.p. 223–225°, (dec.).

Anal. Calcd. for $C_{19}H_{17}N_3O_7$: C, 46.44; H, 3.49; N, 14.25. Found: C, 47.09; H, 3.57; N, 13.81.

Conversion of monofuroyl picrate to monofuroyl hydrochloride. Anhydrous 2.44*M* solution of hydrogen chloride in absolute acetic acid was prepared by dropwise addition with stirring and cooling of 39.3 ml. of acetic anhydride to 10.0 ml. of 12*M* aqueous hydrochloric acid. The monofuroyl picrate (491 mg.) was thoroughly triturated with 2.05 ml. of this solution until the yellow suspended particles had become completely white.

The resulting precipitate was repeatedly washed with small volumes of glacial acetic acid to remove the deep yellow mother liquor, giving 363 mg. (dry weight) of white powder, m.p. about 240° (darkening from 202°). By recrystallization from acetic acid there was obtained 283 mg. (95%) of desiccated (over sodium hydroxide) product, colorless prisms, melting point unchanged. For analysis a sample of this *4-N-furoyl pyridoxamine hydrochloride* was again recrystallized, and vacuum-dried at 60° over NaOH.

Anal. Calcd. for $C_{13}H_{15}ClN_2O_4$: C, 52.26; H, 5.06; N, 9.38. Found: C, 52.20; H, 5.11; N, 9.45.

The product dissolved easily in water over the entire *p*H range. It gave a positive silver nitrate test for chloride, and a positive ferric chloride test for phenolic hydroxyl (deep red color). A sodium nitrite test for free primary amino groups was negative. The spectrum was determined in a potassium bromide pellet with a Perkin-Elmer Model 21 Recording Infrared Spectrometer and showed transmission minima at 3310, 2770, 1600, 1540, 1040, 873, and 757 cm.$^{-1}$

2-Methyl-3-hydroxy-4-furfuryliminomethyl-5-hydroxymethylpyridine (pyridoxal furfurylamine azomethine). To 1.04 g. of commercial pyridoxal hydrochloride in 5.0 ml. of water was added with stirring 0.92 ml. (0.97 g.) of redistilled furfurylamine, b.p. 144–146°, giving a yellow precipitate. To this suspension was gradually (5 min.) added a solution of 530 mg. anhydrous sodium carbonate in 5.0 ml. of water (effervescence). The mixture (*p*H 8–9) was stirred at 90–100° for 10 min., and the resulting nearly clear, deep yellow solution gradually cooled, giving a yellow powder, dry weight 1.25 g. (99%), m.p. 138–140°.

For analysis, 345 mg. was recrystallized from water (75 ml./g.), giving 174 mg. of long, thin, yellow, felted needles with unchanged melting range.

Anal. Calcd. for $C_{12}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.01; H, 5.74; N, 11.08.

The product was very soluble in ethanol, and gave a negative 2,4-dinitrophenylhydrazine test for carbonyl groups (false positive on long standing). A control test with pyridoxal was positive (provided the test tube wall was scratched to initiate crystallization). The infrared spectrum had a transmission minimum at 1630 cm.$^{-1}$, presumably corresponding to C=N stretching vibration.

2-Methyl-3-hydroxy-4-furfurylaminomethyl-5-hydroxymethylpyridine (4-N-furfurylpyridoxamine). To the yellow azomethine (429 mg.) in 10 ml. of water was gradually (30 min.) added 492 mg. of powdered sodium borohydride (effervescence). The now nearly colorless suspension was stirred 2 hr. more, and filtered, giving 200 mg. (40%) of white powder, m.p. 134–135° (mixed melting point with azomethine depressed). Processing of the filtrate failed to yield additional product. For analysis, a sample was recrystallized from acetone, giving colorless needles of unchanged melting point.

Anal. Calcd. for $C_{12}H_{16}N_2O_3$: C, 62.87; H, 6.00; N, 11.28. Found: C, 62.33; H, 6.20; N, 11.81.

The infrared spectrum had a transmission minimum at 1500 cm.$^{-1}$, presumably corresponding to the N—H bending vibration of the secondary amino group, and it lacked the minimum at 1630 cm.$^{-1}$ found in the spectrum of the azomethine.

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(11) The notable change in solubility is evidently not due to formation of a solvate with ethanol. Perhaps it is due to the appearance of a new polymorphic crystalline form; or it may be (as suggested by a referee) that the crude product was a hydrate until treated with absolute ethanol.