

### 1010. *The Effect of Acids on Dihydronicotinamide Derivatives.*

By RUTH SEGAL and GABRIEL STEIN.

The products formed from 1-benzyl-1,6- and -1,4-dihydronicotinamide by addition of the elements of water in acid solution were obtained crystalline and proved to be identical. They have the OH group in the 5-position. These reactions were investigated, and the reaction constants determined. A mechanism is indicated.

BOTH DPNH and TPNH (the reduced forms of coenzymes I and II) are unstable in acid solution,<sup>1</sup> and so are other 1-substituted dihydronicotinamides. Thus it was shown<sup>2,3</sup> that when 1,4- ( $\lambda_{\max}$  340—360 m $\mu$ ) and 1,6-dihydronicotinamides ( $\lambda_{\max}$  340—360, 265—270 m $\mu$ ) were treated with acid, the absorption spectrum changed irreversibly. In both cases a single new peak at 290—300 m $\mu$  appeared, with the simultaneous disappearance of all former peaks.

However, most products formed by the action of acids on dihydronicotinamides could not be crystallized and only few were investigated. Anderson and Berkelhammer,<sup>4</sup> on acidification of 3-acetyl-1-benzyl-1,4-dihydropyridine with dilute hydrochloric acid, obtained a crystalline product which they considered to be 3-acetyl-1-benzyl-1,4,5,6-tetrahydro-6-hydroxypyridine. Wallenfels *et al.*<sup>3,5</sup> acidified the 1,6- and 1,4-dihydro-derivatives of 1-(2,6-dichlorobenzyl)nicotinamide with sulphurous acid, and obtained identical products, formulated as sulphurous acid adducts with two molecules of the dihydropyridine derivatives, the sulphite linkage occupying the 5-positions of the pyridine rings. However, thiophenol gave different addition products with the two dihydronicotinamides.<sup>3</sup>

We have now obtained crystalline products of the action of acids on both 1-benzyl-1,6- and 1,4-dihydronicotinamide and investigated their nature and mode of formation. 1-Benzylnicotinamide has been used<sup>6</sup> as a model compound of DPN<sup>+</sup>.

#### EXPERIMENTAL

1-Benzyl-1,4-dihydronicotinamide was prepared according to directions of Mauserall and Westheimer.<sup>6</sup> The product of the action of acid was prepared from it by dissolving 2 g. in ethanol (40 ml.) and adding water (40 ml.), then 5*N*-hydrochloric acid (1 ml.) and storing the

<sup>1</sup> von Euler, Schlenk, Heiwinkel, and Hogberg, *Z. physiol. Chem.*, 1938, **256**, 208; Warburg and Christian, *Biochem. Z.*, 1934, **274**, 113; Karrer, Schwarzenbach, Benz, and Solmssen, *Helv. Chim. Acta*, 1936, **19**, 811; Karrer, Ringier, Buchi, Fritsche, and Solmssen, *Helv. Chim. Acta*, 1937, **20**, 55.

<sup>2</sup> Paiss and Stein, *J.*, 1958, 2905.

<sup>3</sup> Wallenfels, Hofmann, and Schuly, *Annalen*, 1959, **621**, 188.

<sup>4</sup> Anderson, jun., and Berkelhammer, *J. Amer. Chem. Soc.*, 1958, **80**, 992.

<sup>5</sup> Wallenfels and Schuly, *Biochem. Z.*, 1957, **329**, 75.

<sup>6</sup> Mauserall and Westheimer, *J. Amer. Chem. Soc.*, 1955, **77**, 2261.

whole overnight. Addition of *N*-sodium hydroxide to slight alkalinity precipitated some product and water was added until precipitation was complete. The crystals were filtered off, dissolved in a little chloroform, and reprecipitated with benzene. This last procedure was repeated, affording yellow crystals, m. p. 158—161° (decomp.) (yield 20%) (Found: C, 67.8; H, 6.62; N, 12.1. Calc. for  $C_{13}H_{16}N_2O_2$ : C, 67.2; H, 6.9; N, 12.0%),  $\epsilon_{\max}$   $1.04 \times 10^4$  at 292  $m\mu$ .

1-Benzyl-1,6-dihydronicotinamide was prepared by adapting Wallenfels and Schuly's method.<sup>7</sup> To 1-benzyl-1,6-dihydronicotinamide (2.5 g.) in water (175 ml.) was added with stirring a solution of sodium borohydride (400 mg.) in 2% sodium carbonate-bicarbonate solution (1:1, 75 ml.). The crystalline product showed  $\lambda_{\max}$  356, 268  $m\mu$  ( $\epsilon_{356} = \epsilon_{268} = 4 \times 10^3$ ). It was converted by acid, as in the foregoing case, into a product of m. p. 157—160° (decomp.) (Found: C, 66.8; H, 7.0%).

To test for the presence of the hydroxyl groups we proceeded according to Davidson and Perlman.<sup>8</sup> The acid product (0.1 g.) was dissolved in anhydrous pyridine (0.3 ml.) and added to a mixture of acetyl chloride (0.1 g.) and *NN*-dimethylaniline (0.2 g.). The mixture was heated for 15 min. at 50°, cooled, and mixed with a few grams of ice and then ammonia (*d* 0.880; 1 ml.). After 45 min. it was centrifuged, and the oily layer removed. This was tested for esters by the usual hydroxamic acid method.

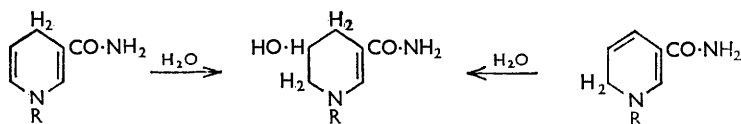
Dinitrophenylhydrazones were prepared by Wild's procedure.<sup>9</sup>

*p*-Nitrobenzoyl derivatives were obtained by boiling the acid product (1 g.) and *p*-nitrobenzoyl chloride (2 g.) in pyridine (20 ml.) for 1 hr., and cooling. The precipitate was washed with dilute aqueous sodium hydrogen carbonate and with water, and recrystallized from hot alcohol; it had m. p. 172° in both cases (mixed m. p. undepressed).

*Kinetic Experiments.*—A solution of the dihydronicotinamide in dilute aqueous sodium hydrogen carbonate was mixed at zero time with an equal volume of buffer solution containing different proportions of 0.2*M*-disodium hydrogen phosphate and 0.1*M*-citric acid. Optical densities at 292 and 360  $m\mu$  were determined at various times on a Beckman D.U. Spectrophotometer at 25°. pH values were determined at the end of the reaction. Velocity constants were obtained by plotting  $\log (D_\infty - D_t)$  against time, ( $D_\infty - D_t$ ) being absolute values.

## RESULTS AND DISCUSSION

On acidification with hydrochloric acid, 1-benzyl-1,6- and -1,4-dihydronicotinamide gave yellow crystals of the same m. p. and  $\lambda_{\max}$  value. Since the mixed m. p. was undepressed, and analysis agrees in both cases with the formula  $C_{13}H_{16}N_2O_2$ , we assume that the products were identical, 1 molecule of water being added to the dihydro-derivatives. As the absorptions of the amidic  $NH_2$  group and of the OH group strongly overlap in the infrared spectrum, this test was not conclusive. However, the presence of the OH group was confirmed by Davidson and Perlman's test.<sup>8</sup> The products gave identical crystalline *p*-nitrobenzoyl derivatives. The absorption spectra of the various compounds have been considered previously.<sup>4,10</sup> We may attribute the absorption band of the acid product at 290  $m\mu$  to the conjugated system  $N=C=C=O$ .<sup>7</sup> One possibility for obtaining identical products is by addition of water:



In this case the OH group will occupy the 5-position. Alternatively, an acid-catalysed rearrangement of one of the dihydro-derivatives to the other isomer may be followed by addition of water: in this case the OH group could be in the 4-, 5-, or 6-position.

<sup>7</sup> Wallenfels and Schuly, *Annalen*, 1959, **621**, 106.

<sup>8</sup> Davidson and Perlman, "A Guide to Qualitative Organic Analysis," Brooklyn College, N.Y., 1958.

<sup>9</sup> Wild, "Characterization of Organic Compounds," Cambridge Univ. Press, 1958, p. 114.

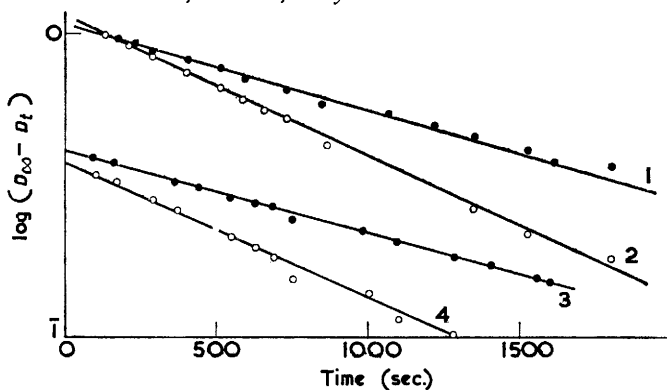
<sup>10</sup> Cilento, de Carvalho Filho, and Giora Albanese, *J. Amer. Chem. Soc.*, 1958, **80**, 4472.

We therefore investigated the kinetics of the formation of the product from both isomers. Within the rather narrow pH range (5.2—5.8) where the reaction velocity was amenable to experiment, the rate of adduct formation was measured spectrometrically. The Figure shows a representative set of measurements. Identical rate constants were obtained by following the rate of formation at 292  $m\mu$  and the rate of disappearance at 360  $m\mu$ . The pseudo-first-order rate constants,  $k$ , and the second-order rate constants,  $k' = k/C_{H^+}$ , are shown in the Table at three pH values.

pH	1,6-Dihydro-compound		1,4-Dihydro-compound	
	$10^3k$ (sec. <sup>-1</sup> )	$10^{-2}k'$ (sec. <sup>-1</sup> mole <sup>-1</sup> )	$10^3k$ (sec. <sup>-1</sup> )	$10^{-2}k'$ (sec. <sup>-1</sup> mole <sup>-1</sup> )
5.2	1.9	3.2	3.4	6
5.4	1.26	3.16	2.44	6.1
5.82	0.62	4.05	1.03	6.3

It is seen from the linear first-order plot (at 292  $m\mu$ ) for the formation of the product of acidification, that the conversion of both dihydro-derivatives does *not* include relatively

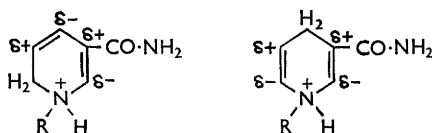
*The logarithm of the absolute value of ( $D_\infty - D_t$ ) as a function of time in the reactions of 1-benzyl-1,4- and -1,6-dihyronicotinamide.*



(1) 1,6-, at 292  $m\mu$ ; (2) 1,4-, at 292  $m\mu$ ; (3) 1,6-, at 360  $m\mu$ ; (4) 1,4-, at 360  $m\mu$ .

slow consecutive reactions. The different rate constants for the disappearance of the 1,6- and 1,4-derivatives exclude a fast isomerization which would precede the addition of water.

Anderson and Berkelhammer<sup>4</sup> interpreted their results as showing that 3-acetyl-1-benzyl-1,4-dihydropyridine yields an acid product on which the OH group is in the 6-position. We have tested for this possibility by trying to convert our products into the 2,4-dinitrophenylhydrazones by treatment with acid 2,4-dinitrophenylhydrazine. Were the hydroxyl group in the 6-position, the aldehyde ammonia function would yield a phenylhydrazone, but the precipitate obtained had neither the typical spectrum of



2,4-dinitrophenylhydrazones nor the typical shift in spectrum in alkaline alcoholic solutions.<sup>11</sup> These experiments further support the view that in our case the hydroxyl group is unlikely to be in the 6-position and is in the 5-position.

<sup>11</sup> Timmons, *J.*, 1957, 2613; Johnson, *J. Amer. Chem. Soc.*, 1953, **75**, 2720.

If the reaction proceeds by a primary proton attack, identical water adducts are to be expected from both nicotinamides: in both cases partial positive charges would reside on the 5-carbon atom, on which hydroxyl addition will take place, as shown. The partial positive charge on the 5-position is weakened for the 1,6-isomer by the effect of the amide oxygen. The lower velocity constant in this case is consistent with this.

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HEBREW UNIVERSITY, JERUSALEM, ISRAEL.

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