

Table 1. PROPERTIES OF DIPHOSPHOPYRIDINE NUCLEOTIDE AND OF ITS NICOTINIC ACID ANALOGUE

Property	Nicotinic acid analogue of DPN	DPN*
$R_{adenine}$ in ethanol-1 M ammonium acetate, pH 7.5 (70:35, v/v)	0.23	0.26
R_F in isobutyric acid-1 N ammonia (100:60, v/v)	0.33	0.47
Relative electrophoretic mobility in 0.1 M ammonium acetate, pH 5 at 25 V/cm.	1.5	1.0
Ultra-violet absorption at pH 7:		
maximum (m μ)	258	259
minimum (m μ)	230	231
$E_{max.}: E_{min.}$	2.19	2.22
$E_{250}: E_{260}$	0.86	0.83
$E_{270}: E_{260}$	0.74	0.72
$E_{280}: E_{260}$	0.28	0.22
$\epsilon \times 10^{-3}$	18.75 \pm	18.00 \pm
	17.15 \S	
Ultra-violet absorption of cyanide complex		
maximum of 1st peak (m μ)	260	260
maximum of 2nd peak (m μ)	316	327
minimum (m μ)	289	289
$E_{max.}$ of 1st peak: $E_{min.}$	6.4	7.6
$E_{max.}$ of 1st peak: $E_{max.}$ of 2nd peak	3.4	2.5
$E_{250}: E_{260}$	0.75	0.85
$E_{270}: E_{260}$	0.75	0.74
$E_{280}: E_{260}$	0.30	0.27

* Spectral data for diphosphopyridine nucleotide (DPN) are taken from the literature (ref. 9).

† From Kornberg and Horecker (ref. 3).

‡ Based on ribose analysis.

§ Based on phosphate analysis.

|| Figures for the cyanide complex of the analogue are calculated from the ultra-violet spectrum determined in 30 per cent (v/v) ethanol.

derivatives other than nicotinamide^{2,4}. Anion exchange chromatography ('Dowex-1' formate, gradient elution with formic acid⁵) of the products obtained after incubation of diphosphopyridine nucleotide and nicotinic acid with the beef-spleen enzyme showed four peaks. These corresponded to the following compounds, listed in the order they emerge from the column: nicotinamide, nicotinic acid and diphosphopyridine nucleotide, the new analogue of diphosphopyridine nucleotide, and adenosine diphosphate-ribose. The analogue was eluted from the resin in the same position as the product isolated from the mould: furthermore the two compounds were indistinguishable by paper chromatography (two different solvents) and by paper electrophoresis at pH 5, and had identical spectral properties. The chromatographic, electrophoretic and spectral properties of the new pyridine nucleotide and of diphosphopyridine nucleotide are reported in Table 1.

The presence of a carboxyl group in the molecule of the new pyridine nucleotide increases the negative charge of the molecule, as reflected in the electrophoretic mobility of the compound, which is 1.5 times that of diphosphopyridine nucleotide, and in the previously mentioned behaviour in anion-exchange chromatography. The carboxyl group is probably responsible also for the decreased reactivity of the new compound with cyanide: in fact the rate of the reaction with cyanide was quite slow in aqueous solution, whereas in 30 per cent ethanol the peak at 316 m μ appeared very rapidly. In this respect it is interesting to note that N'-methyl-nicotinic acid (trigonellin) does not react with aqueous cyanide⁶ but will react in alcoholic solution⁷, in contrast to N'-methyl-nicotinamide, which reacts with aqueous cyanide⁶. These two model substances are structurally interrelated in the same way as diphosphopyridine nucleotide and its nicotinic acid analogue.

At present no data are available on the biological significance of the new pyridine nucleotide. We are now investigating its possible role as an intermediate

in synthesis of diphosphopyridine nucleotide from nicotinic acid by human erythrocytes. Recently, Preiss and Handler⁸ have suggested that in this process "amidation may occur after nicotinic acid is converted to some unknown nucleotide derivative".

We thank Prof. E. B. Chain for his interest in this investigation.

Note added in proof. We have now isolated from human erythrocytes incubated with nicotinic acid a compound identical with the new pyridine nucleotide from *P. chrysogenum*¹⁰. This shows that one of the intermediates ('compound II') found by Preiss and Handler in the synthesis of diphosphopyridine nucleotide from nicotinic acid in human erythrocytes¹¹ is, in fact, the nicotinic acid analogue of diphosphopyridine nucleotide, that is, desamido-diphosphopyridine nucleotide, as they suggested.

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Fungistatic Activity of some Hydroxamic Acids

It was found by two of us¹ that salicylhydroxamic acid completely inhibits growth of some pathogenic fungi (*Trichophyton schoenleinii*, *T. gypseum*, *T. rubrum*, *T. violaceum*) *in vitro* at a concentration of 0.025–0.1 per cent (0.25–1.0 mgm. per 1 ml.). A similar effect was produced by benzohydroxamic acid and 2-hydroxy-3-naphthhydroxamic acid². It was interesting to find that 5-bromosalicylhydroxamic acid ('T 40')³ (which has now found clinical application in Poland as an auxiliary remedy in treating tuberculosis in addition to 'INH')⁴ possesses a very low fungistatic activity.

Salicylhydroxamic acid, which formerly was found to be of low toxicity⁵, was clinically examined at the Municipal Hospital No. 1 in Poznan. From the beginning of 1956 to the middle of 1957 twenty-one cases of trichophytiasis and one case of epidermophytiasis were examined. Salicylhydroxamic acid was given *per os* during 10 days in doses 1 gm. and 0.5 gm. daily to adults and children, respectively. Quick healing followed, which in many cases was very spectacular. Simultaneously, systematic investigation of blood and urine of patients did not show any unfavourable effect due to the treatment. Salicylhydroxamic acid might therefore be a possible remedy against diseases produced by pathogenic fungi. Detailed description of clinical experiments will be published by one of us (J. A.) elsewhere.

Another line of research consisted in preparing a number of derivatives of phenoxyacethydroxamic acid and determining their fungistatic activity against *Fusarium culmorum*, *Alternaria solani* and *Rhizoctonia solani*⁶. Chlor-derivatives of phenoxyacethydroxamic acid proved to exert a particularly strong fungistatic activity *in vitro*.

Thus 4-chloro-2-methylphenoxyacethydroxamic acid was effective against *F. culmorum* at a concentration 0.005 per cent in solid agar culture medium. 2,4-Dichlorophenoxyacethydroxamic acid was effective against all three fungi examined at concentration 0.025 per cent. Similar activity was shown by 3,4-, 2,5-di- and 2,4,6-trichloro-derivatives.

The same group of compounds was examined against pathogenic fungi. 3,4-Dichlorophenoxyacethydroxamic acid was found to be particularly effective not only against *Trichophyton* but also against various pathogenic yeasts. It completely inhibited growth of *Candida albicans*, *C. krusei*, *C. tropicalis*, *Cryptococcus neoformans*, *Geotrichum 1* and *Geotrichum malatensis 53* when used in concentration 0.003–0.025 per cent in liquid Sabouraud medium.

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Ethyl-3-Indoleacetate: an Artefact in Extracts of Immature Corn Kernels

THE isolation and identification of 3-indoleacetic acid in corn kernels has been well established¹. While characterization of ethyl-3-indoleacetate in ethanol extracts of corn kernels, *Zea mays*, variety Golden Cross, has been reported from this laboratory², it has been suggested that ethanol extraction may have resulted in the formation of an artefact³.

To avoid esterification, peroxide-free ethyl ether was used as the extractant in the present study. Freshly harvested, immature (early milk stage) corn kernels, variety Golden Cross, were covered with ethyl ether and extracted for two hours at 2° C. ('free auxin' or first fraction). Additional cold ethyl ether washings of the kernels were added to the original extract. The kernels were again covered with ethyl ether and extracted for 48 hr. at 25° C. ('bound auxin' or second fraction). The first and second ethyl ether fractions were further separated into

acidic and non-acidic ('neutral') portions by extraction with 5 per cent aqueous sodium bicarbonate. The bicarbonate solution containing the acidic substances was adjusted to pH 2.8 with hydrochloric acid and extracted with ethyl ether. The aqueous layer was discarded, and the acidic substances in the ether layer were retained. Acidic and non-acidic ethereal solutions were separately concentrated under reduced pressure with the bath temperature kept below 25° C. The constituents of the concentrated extracts were partitioned by paper chromatographic techniques (Whatman No. 1 filter paper, solvent mixture 2-PrOH:NH₃:H₂O (8:1:1 v/v)⁴) and their biological activities assayed⁵.

3-Indoleacetic acid was detected in the chromatographically separated acid fractions through biological assays, by development of characteristic colours with Salkowski and Ehrlich spray reagents, and ultra-violet absorption spectra. In addition, growth-promoting zones with R_F 0.25–0.33 and growth-inhibiting zones with R_F 0.60–0.70 were noted in both acid fractions. A similar observation of growth-stimulating and -inhibiting zones has been reported for absolute ethanol extracts of corn kernels (ref. 1, Kefford).

Growth-stimulating zones of R_F 0.80–0.90 were observed in histograms of neutral fractions. The R_F values were similar to those reported for ethyl-3-indoleacetate and 3-indoleacetonitrile; however, the presence of an indole moiety could not be established by ultra-violet absorption spectra or by the reactions to Salkowski and Ehrlich colour reagents. Absence of ethyl-3-indoleacetate in the ethyl ether extracts of corn kernels, *Zea mays*, variety Golden Cross, indicated that it was previously isolated as an artefact². The characterization of this neutral growth substance is now in progress, and a more detailed report will be published elsewhere.

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Actinomycete Disintegration of Raw Wool

DURING the course of investigations on bacterial disintegration of raw wool, the presence of Actinomycetes in rotted wool was often noted. These organisms, however, rarely appeared in culture, perhaps because of the prevalence of *Pseudomonas aeruginosa* the antagonistic effects of which are well known. Eventually Actinomycetes were isolated