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STUDIES OF THE RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND PORPHYRIA-INDUCING ACTIVITY*

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Abstract—A porphyria, induced in guinea pigs by means of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC) is known to be the result of an enhanced synthesis of the enzyme, δ-aminolaevulic acid synthetase in liver cells. To further examine this phenomenon several analogues of DDC (Ib) and the corresponding pyridine (IIb) have been prepared and tested for porphyria-inducing activity. A study of the Fisher-Hirschfelder-Taylor models and the u.v. absorption spectra of these analogues indicated that the 2-, 4-, and 6-methyl substituents cause a twisting of the 3- and 5-ethoxycarbonyl substituents out of the plane of the ring. The nonplanar relationship between the ethoxycarbonyl substituents and the ring appears to be necessary for optimal porphyria-inducing activity

DRUG-INDUCED changes in the porphyrin metabolism of experimental animals, resembling those seen in acute intermittent porphyria, were first observed in 1952 with allylisopropylacetylcarbamide (Sedormid)[1] and subsequently with barbiturate derivatives.[2] These drugs are closely related in structure, and a study of structure–activity relationships[3–5] in this series revealed a high degree of structural specificity for porphyria-inducing activity. The recent discovery of a series of structurally unrelated porphyria-inducing drugs was therefore unexpected.[6–8]

The studies of Granick and Urata[7, 8] showed that the overproduction of porphyrins and porphyrin precursors in the livers of animals fed porphyria-inducing drugs resulted from an enhanced synthesis of the first enzyme in the porphyrin biosynthetic pathway, *viz.*, δ-aminolaevulic acid synthetase. On the basis of these studies, Granick and Levere[9] suggest that there is a mechanism for the control of this enzyme by repression of its formation and that the porphyria-inducing drugs interfere with the repressor control mechanism. An important question that remains to be answered concerns the nature of the cellular receptor with which the porphyria-inducing drugs interact to interfere with the repressor control mechanism. Recent studies of the molecular nature of drug-receptors are based on the assumption that small molecules whose biological action is specific and structure-dependent have a molecular structure complementary to the site at which they act. A study of the structure–activity relationship of porphyria-inducing drugs is therefore a means with which the chemical nature of the drug-receptor may be explored. This consideration has led us to undertake further studies of the structure–activity relationship in porphyria-inducing drugs.

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Table 1. Dihydropyridine and pyridine derivatives

Compound no.	Compound	Reference	m.p., °C	b.p., °C	Ultraviolet spectrum						Infrared spectrum cm^{-1}		
					λ max	$\epsilon \times 10^{-3}$	λ max	$\epsilon \times 10^{-3}$	λ max	$\epsilon \times 10^{-3}$	Secondary amine	Carbonyl group	Pyridine ring
Ia	3,5-Diethoxycarbonyl-1,4-dihydro-2,6-dimethylpyridine	13	175–184		231	16·0	373	7·0			3,325	1,680	
Ib	3,5-Diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine	15	128·5–129		232	18·2	351	8·05			3,320	1,690	
Ic	3,5-Diethoxycarbonyl-1,4-dihydro-2,6-dimethyl-4-ethylpyridine	16, 15*	108–110		233	16·0	349	7·41			3,320	1,690	
Id	3,5-Diethoxycarbonyl-1,4-dihydro-2,6-dimethyl-4-propylpyridine	15,* 17	116–120		235	16·2	349	7·25			3,350	1,700	
Ie	3,5-Diethoxycarbonyl-1,4-dihydro-2,6-dimethyl-4-phenylpyridine	18	156·5–157·5		239	19·08	356	7·9			3,310	1,680	
IIIa	3,5-Diacetyl-1,4-dihydro-2,6-dimethylpyridine	20	197–208		253	10·65	277·5	6·51	409	7·05	3,320	1,660	

TABLE 1.—*continued*

IIIb	3,5-Diacetyl-1,4-dihydro-2,4,6-trimethyl-pyridine	20,† 21	152–154	150–168/ 0·25 mm	255	15·4	380	9·16			3,310	1,675	
IIa	3,5-Diethoxy-carbonyl-2,6-dimethylpyridine	14	71–72·5		235	11·4	273	3·94	281	3·24		1,715	1,585
IIb	3,5-Diethoxy-carbonyl-2,4,6-trimethylpyridine	14,‡ 19		138/ 0·4 mm	270	3·25						1,715	1,565
IIc	3,5-Diethoxy-carbonyl-2,6-dimethyl-4-ethylpyridine	14,‡ 16		130/ 0·45 mm	269	3·38						1,715	1,560

* Acetaldehyde was replaced by propionaldehyde in the synthesis of Ic and by butyraldehyde in the synthesis of Id.

† A modification of the procedure used for the synthesis of IIIa was employed for the synthesis of IIIb. Formaldehyde was replaced by acetaldehyde and, after saturating the solution with ammonia, the ethanol was removed and the residue distilled. Trituration of the oil obtained, with ether, yielded orange-yellow crystals which were crystallized from ethanol.

‡ For the preparation of IIb and IIc the reaction time was increased to 12 hr and the oily products collected with ether and distilled.

Since 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC) is the most active of the porphyria-inducing drugs† it was chosen as the starting point for our investigation, and in this paper a study of structure–activity relationships of analogues of DDC is described.

EXPERIMENTAL

Ultraviolet absorption spectra were determined in absolute ethanol, in a Bausch and Lomb Spectronic 505 spectrophotometer. The infrared spectra were obtained with a Perkin Elmer 137 sodium chloride spectrophotometer; solid compounds were mulled with Nujol, and liquid samples were used as liquid films. Melting points are uncorrected.

Induction of porphyria in guinea pigs. Male or female guinea pigs were placed in metabolic cages allowing separate collection of urine and faeces. To induce a porphyria the guinea pigs were deprived of food on the first day, and during the following two days were fed the drug under investigation once daily by gastric intubation. Solid drugs were suspended in 0·25 M sucrose (1 g/12·5 ml) for intubation, and liquid drugs were intubated directly. The weight of the animals and the dose of drug used in each experiment are recorded in Table 1.

Determination of δ-aminolaevulic acid and porphobilinogen in urine. δ-Aminolaevulic acid and porphobilinogen were determined in 24-hr samples. These metabolites were separated by ion-exchange chromatography and estimated according to the procedure of Mauzerall and Granick.[10]

Synthesis of compounds tested for porphyria-inducing activity. For the synthesis of pyridine and dihydrophyridine compounds, standard methods or modifications of standard methods were used. Details of the melting points, boiling point and u.v. and i.r. spectra are recorded in Table 1.

For synthesis of diethyl β-methyl glutarate and diethyl α-methylsuccinate, the di-esters were prepared by heating the corresponding di-acids with ethanol containing sulphuric acid. Diethyl β-methylglutarate had b.p. 118°/14 mm; diethyl α-methylsuccinate b.p. 102°/14 mm. Karrer and Lee[11] record b.p. 121–122°/16 mm for diethyl β-methylglutarate and Wojcik and Adkins[12] record b.p. 108–110°/16 mm for diethyl α-methylsuccinate.

RESULTS AND DISCUSSION

The ability of DDC analogues to induce an increased synthesis of δ-aminolaevulic acid synthetase in guinea pig liver was determined by feeding these compounds to guinea pigs and estimating the amount of δ-aminolaevulic acid and porphobilinogen excreted in the urine (Table 2).

Studies of structure–activity relationships of porphyria-inducing chemicals in the whole animal suffer from the fact that a distinction cannot be drawn between the effects of structural variations at the site of action and on the dynamic phenomena (absorption, distribution, metabolic destruction, and excretion) that control drug concentration at that site.[22] This distinction can be drawn by means of a recent elegant method developed by Granick.[8] The method consists in adding porphyria-inducing chemicals to chick embryo liver cells, cultured on cover slips, and measuring the

† S. Granick, personal communication ; G. S. Marks and D. Schneck, unpublished observations.

TABLE 2. ANALYSES OF 24- AND 48-HOUR URINE OF GUINEA PIGS FED DDC OR ANALOGUES FOR 2 DAYS*

The extreme values are given in parentheses.

Compound no.†	No. of animals used	Average wt. of animals (g)	Amt. of drug fed/ day/2 days (g)	Component in 0 to 24-hr urine		Component in 24 to 48-hr urine	
				δ-Aminolaevulic acid (μmoles)	Porphobilinogen (μmoles)	δ-Aminolaevulic acid (μmoles)	Porphobilinogen (μmoles)
Ia	3	413 (390–425)	0·75	0·81 (0·4–1·1)		0·90 (0·3–1·7)	
Ib	3	354 (326–437)	0·95	0·9 (0·8–0·9)	1·6 (0·2–2·8)	0·9 (0·7–1·3)	1·8 (0·2–3·0)
Ic‡	5	433 (416–446)	0·75	2·1 (0·1–5·0)	3·1 (0·3–5·4)	6·5 (1·6–9·9)	14·6 (10·3–17·7)
Id	5	662 (600–725)	0·75	1·07 (0·27–3·4)	2·8 (0·35–7·1)	14·4 (0·7–33·4)	25·7 (16·5–49·3)
Ie	5	329 (316–345)	0·4	0·08 (0·04–0·13)		0·08 (0·04–0·13)	
IIIa	3	415 (372–466)	0·75	1·3 (0·6–1·8)		0·7 (0·5–0·8)	
IIa	4	376 (320–414)	0·75	0·08 (0·04–0·13)		0·30 (0·12–0·53)	
IIb	4	434 (367–514)	0·6	0·5 (0·1–0·9)		0·7 (0·1–1·4)	
IIc	5	673 (585–730)	0·52	0·15 (0·03–0·25)		1·4 (1·1–2·0)	

* In the 0 to 24-hr urine of 3 normal guinea pigs, δ-aminolaevulic acid 0·08 μM (0·04–0·12) was found; porphobilinogen could not be detected.

† See Table 1 for compounds.

‡ A marked tremor and weakness of the hind legs was observed in the guinea pigs. Later they became very weak and shivered violently, owing to a marked lowering of body temperature.

porphyrin accumulation by means of fluorescence microscopy. We are greatly indebted to Dr. Granick for having supplemented our studies in guinea pigs with tests of the DDC analogues in his *in vitro* system. The results obtained by Dr. Granick are recorded in Table 3.

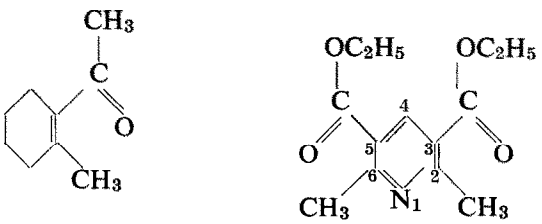
TABLE 3. PORPHYRIN ACCUMULATION IN CHICK EMBRYO LIVER CELLS, INDUCED BY DDC AND STRUCTURAL ANALOGUES AND MEASURED BY FLUORESCENCE MICROSCOPY

The liver from a 17-day-old chick embryo was treated with trypsin to obtain a cell suspension. Approximately 3×10^5 liver parenchyma cells were inoculated into a vial (18 × 60 mm) containing a cover slip and 1 ml of Eagle's medium supplemented with 10% bovine foetal serum (Microbiological Associates Inc.). After 24 hr of growth in an atmosphere of 5% CO_2 in air, at 37°, the medium was replaced by 1 ml of fresh medium, and DDC or structural analogue dissolved in 1λ of ethanol was added. Fluorescence intensity was scored as follows: +3, most colonies fluoresce intensely; +2 most colonies fluoresce partially; +1 some colonies fluoresce partially (cf. Granick[8]).

Compounds tested for porphyria-inducing activity in chick-liver parenchyma cells	Concentration $M \times 10^{-6}$	Intensity of fluorescence observed 24 hr after addition of compound
Ia	8	0
	40	0·5
Ib	7·5	3
	3·8	1
	1·4	+trace
	0·75	+trace
	0·38	0
Ic	7·2	2
	3·6	2
	0·72	0·75
	0·072	+trace
Id	6·8	1·75
	0·68	0·25
	0·32	0·25
	0·16	0
IIIa	10·4	0
	52	0
IIIb	9·7	0
	49	0
IIa	8	0
	40	0·5
IIb	7·5	1·5
	3·8	1
	1·9	+trace
	0·75	0
IIc	7·2	2
	3·6	1
	1·9	+trace
	0·72	0

* See Table 1 for compounds.

The first analogue investigated, 3,5-diethoxycarbonyl-1,4-dihydro-2,6-dimethylpyridine (Ia), differed from the parent, dihydropyridine (Ib), by the replacement of the 4-methyl substituent with a hydrogen atom. This modification in structure results in a considerable loss in activity (Tables 2 and 3). A similar effect was noted in the corresponding pyridine compounds IIa and IIb (Tables 2 and 3). It is noteworthy that although the pyridine IIb, previously reported to be inactive,[23] was only weakly active *in vivo* (Table 2), considerable activity was found in the *in vitro* system (Table 3). This difference, which is probably due to the influence of dynamic phenomena on drug concentration at the site of action, demonstrates the superiority of the *in vitro* method of testing for porphyria-inducing activity.



(II) (I)

FIG. 1. s-*Cis* configuration (II) of 2-methyl-acetylcyclohexene and the planar configuration (I).

Since 2-methyl-acetylcyclohexene[24] exists in the s-*cis* configuration (II) (Fig. 1) it is probable that pyridine IIa, in which the ethoxycarbonyl substituents are conjugated with the ring, will adopt the planar configuration (I). Inspection of Fisher-Hirshfelder-Taylor models of pyridines IIa and IIb reveals that a 4-alkyl substituent causes a twisting of the ethoxycarbonyl substituents out of the plane of the ring. This steric effect results in an inhibition of resonance which is reflected in the u.v. absorption spectra (Table 1) of pyridines IIb and IIc by a lowered extinction value and by a shift of the absorption maxima to shorter wavelengths[25] relative to pyridine IIa. The u.v. spectral data (Table 1) indicate that the high activity of the 4-alkyl-substituted dihydropyridines Ib, Ic, and Id relative to the dihydropyridine Ia is also due to the steric effect of the 4-alkyl substituents. Apparently a requirement for optimal activity in these compounds is a nonplanar relationship between the ethoxycarbonyl substituents and the pyridine or dihydropyridine ring.

The following observations support the idea that specifically oriented ethoxycarbonyl substituents are necessary for maximal activity. (1) The dihydropyridines IIIa and IIIb, containing acetyl substituents at positions 3- and 5- in place of ethoxycarbonyl substituents, are inactive. (2) Diethyl β-methylglutarate, which can adopt a configuration such that the orientation and distance between its two ethoxycarbonyl substituents is the same as in the active pyridines and dihydropyridines, has a low but definite activity in the *in vitro* system. On the other hand diethyl α-methylsuccinate, in which the distance between the two ethoxycarbonyl substituents is less than that existing in the dihydropyridines and pyridines, is inactive. It is likely that removal of the 2- and 6-methyl substituents of dihydropyridine Ib and pyridine IIb would result in co-planarity of the ethoxycarbonyl substituents with the ring and therefore in reduced activity. Experiments designed to test this idea are in progress.

The experiments in guinea pigs indicated that the probable order of biological activity of the dihydropyridines was Id > Ic > Ib ⩾ Ia, whereas the *in vitro* experiments indicated the order Ic > Id > Ib ⩾ Ia. Interpretation of the *in vitro* experiments is not complicated by dynamic phenomena and reflects more accurately on the activity of these molecules at the receptor. The dihydropyridine Ie, which has a 4-phenyl substituent, is inactive (Table 2). This indicates the necessity for a substituent in the 4-position large enough to exert a steric effect on the 3- and 5-ethoxycarbonyl substituents but not so large as to inhibit interaction of the molecule with a receptor.

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