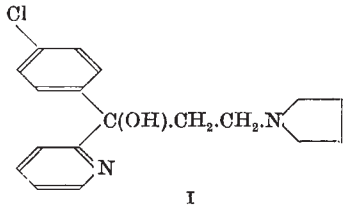
Geometrical Isomers in a Series of Antihistamines

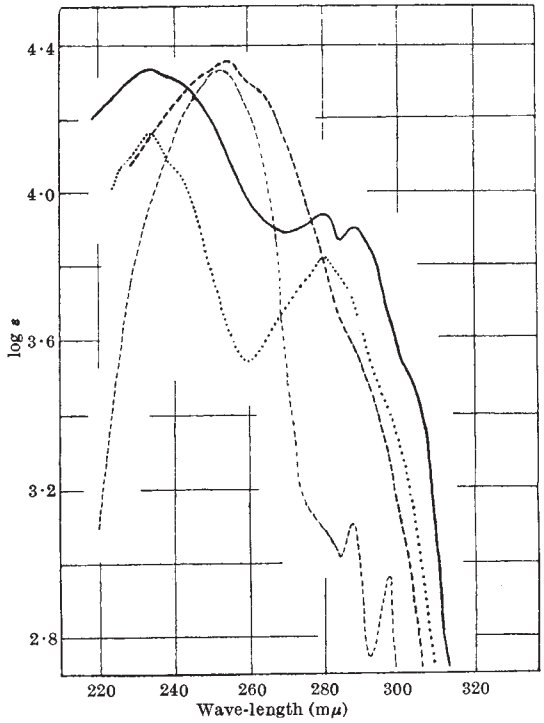
The dehydration of a series of 3-tertiaryamino-1-phenyl-1-α-pyridylpropan-1-ols (for example, I) by heating with 85 per cent sulphuric acid was described recently[1].



I

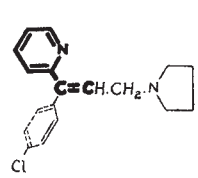
The allylamines so produced are of interest as antihistamines, in particular 1-*p*-chlorophenyl-1-α-pyridyl-3-pyrrolidinoprop-1-ene, also known as 405*C*49, which is among the most potent of known antihistamines. Further study of the preparation of this compound has revealed the presence of an isomeric base (496*C*50) in the product of dehydration. The isomers were separated by fractional crystallization of their oxalates (405*C*49 base, m.p. 61–62° ; oxalate, m.p. 184° (decomp.) ; 496*C*50 base, oil ; oxalate, m.p. 156–157° (decomp.)). The bases are apparently geometrical isomers, for each gave a high yield of the same ketone (*p*-chlorophenyl α-pyridyl ketone, m.p. 63°) on oxidation with chromic acid, and the same propylamine (n_D^{19}, 1·570 ; oxalate, m.p. 147°), when catalytically hydrogenated.

The ultra-violet absorption spectra of the isomers are remarkably distinctive, the one shown by 405*C*49 being almost identical with that of α-pyridylethylene, while that of 496*C*50 closely resembles that of *p*-chlorophenylethylene (see graph). We interpret these

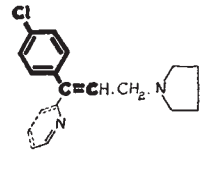


Ultra-violet light absorption spectra. Ordinate, logarithm of molecular extinction coefficient ; abscissa, wave-length in mμ. ——, 405*C*49 ; ...., α-pyridylethylene ; – – –, 496*C*50 ; – · –, *p*-chlorophenylethylene

spectra as indicating that in 405*C*49, as in α-pyridylethylene, the α-pyridyl and ethylenic groups are approximately co-planar, on the generally accepted assumption that this configuration is required for maximal electronic interaction of the two groups to which the characteristic light absorption is related. By the same reasoning, the *p*-chlorophenyl group of 496*C*50, rather than the α-pyridyl group, is in the same plane as the ethylenic group. These configurations are represented in formulæ II (405*C*49) and III (496*C*50), in which the approximately co-planar groups are heavily outlined. Chemical and physical evidence is being sought to determine the relative configuration (*cis-* or *trans-*) of the aminomethylene moiety : it was not possible to make a decision on this point from examination of available molecular models.



II



III

Further, outstanding differences in chemical behaviour of the isomers have been observed. For example, 405*C*49 can be distilled unchanged under reduced pressure, whereas 496*C*50 suffers decomposition ; 405*C*49 is unaffected by boiling acetic anhydride, whereas 496*C*50 is degraded to a mixture of products of as yet undetermined constitution. We suggest that these differences in chemical reactivity may be attributed to the different resonance systems preponderating in the isomers.

The dehydration of many related α-pyridyl carbinols has been re-investigated. In each case the results were similar to the example quoted above : two isomers were formed, one being chemically stable and exhibiting the α-pyridylethylene type of spectrum, the other being relatively unstable and having a spectrum similar to the corresponding substituted phenylethylene. Separation of the isomers was usually effected by fractional crystallization of the oxalates or by chromatography, the progress of the separation being followed by measurements of the ultra-violet absorption spectra. The degradation of the unstable (phenylethylene type) isomer by acetic anhydride provided a convenient method for isolating the stable (α-pyridylethylene type) isomer free from the former.

The pharmacological properties of the isomers differ in an interesting manner. High and specific antihistamine activity was shown only by the isomers having the α-pyridylethylene type of structure, the other isomer of each pair invariably being considerably less active in this respect. In contrast, other pharmacological activities were approximately of the same low order in both isomers. For example, 405*C*49 is about eighty times more potent than 496*C*50 when tested on histamine-induced spasm of guinea pig ileum ; but the two compounds show only minor differences in antagonism of acetylcholine spasm in guinea pig ileum, corneal anæsthetic activity in rabbits or toxicity in mice.

This series of isomers thus provides an illustration of the selective influence of spatial configuration upon chemical and biological reactivity. We are following up the more fundamental implications of these pre-

liminary results and intend to examine the application of these concepts to related compounds.

A more detailed description of the present work will be published elsewhere.

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[1] Adamson, D. W., and Billinghurst, J. W., *J. Chem. Soc.*, 1039 (1950).

Oxygen Uptake of Nucleated and Non-nucleated Halves of *Amœba proteus*

The removal of the nucleus causes a slow decrease of the ribonucleic acid content of the cytoplasm of *Amœba proteus*[1]. This indicates that the ribonucleoprotein particles (microsomes) subsist in the cytoplasm only in the presence of the nucleus. However, this nuclear control is not immediate, for the ribonucleic acid content of non-nucleated fragments drops significantly only after the fourth day following enucleation.

It appeared of interest to find out whether the oxygen uptake is under nuclear control; a rapid decrease of respiration after enucleation, as stated by Clark[2], might mean that respiratory enzymes, known to be predominantly bound to mitochondria, are dependent on the nucleus for the maintenance of their activity.

Measurements were made on nucleated and non-nucleated fragments of *Amœba proteus*, cut into two approximately equal parts with the aid of a glass needle, and kept fasting; a cylindrical Cartesian diver (gas volume approximately 7 c.mm.) was used. Preliminary experiments indicated that oxygen uptake is proportional to the number of amœbæ when 10–30 amœbæ per diver are studied. We found an average value of $0{\cdot}3 \times 10^{-3}$ c.mm./hr./whole amœba; this figure is almost one-fifth of that given by Clark[2] ($1{\cdot}4 \times 10^{-3}$ c.mm.).

Sixty-nine measurements were performed, using 50–100 nucleated and non-nucleated halves; the time interval after enucleation varied from 1 to 10 days and respiration was studied during 4–5 hr. Duplicate experiments gave consistent results within ± 10 per cent.

As Holter and Zeuthen[3] have shown for intact amœbæ (*Chaos chaos*), the oxygen uptake decreases progressively during fasting and a similar phenomenon was found in the present experiments. But, as is shown in Table 1, the ratio between oxygen uptake of nucleated and non-nucleated fragments remains constant for at least nine days, and the uptake of the enucleated fragments 35–45 per cent that of the total.

In order to check if the oxygen uptake is proportional to the protein content of the two fragments, we determined their tyrosine content according to the principle adopted by Andresen and Holter[4]; we followed detailed instructions kindly sent to us by Dr. Holter. The equal intensity of the Millon reaction in both types of fragments[1] justifies the choice of this amino-acid. As is shown in Table 2, results of these determinations expressed as the ratio between both fragments are reasonably of the same order as those found for oxygen uptake.

It can thus be concluded from the results that: (1) the oxygen uptake due to the nucleus of the amœba must be proportionally small; (2) the absence of nucleus does not prevent the prolonged maintenance of a normal level of oxidation. It then seems probable that the respiratory enzymes bound to mitochondria are largely independent of the nucleus for the maintenance of their activity.

It has recently been shown by Mazia and Hirshfield[5] that phosphorus-32 uptake by amœbæ is strongly dependent on the presence of the nucleus: after twenty-four hours, enucleation results in the ratios nucleated : enucleated varying from 3·1 to 6·5.

Table 1

Day after sectioning	1	2	4	5	6	7	8	9	10
Ratio between respiration of nucleated and of non-nucleated fragments (*N*/*E*)	1·36 1·26	1·49 1·49	1·48 1·63	1·26	1·47	1·42	1·35 1·22	1·32	1·86 1·71
Oxygen uptake of enucleated fragments as per cent of total	41 44	40 40	43 38	44	40	41	42·5 45	45	35 40

Table 2

Day after sectioning	1	2	3	8	10
Ratio between tyrosine content of nucleated halves and of non-nucleated halves (*N*/*E*)	1·12 1·22	1·29 1·50	1·42	1·78 1·44	1·84

One is therefore led to believe that enucleation leads to a break in the normal coupling between oxidations and phosphorylations: the non-nucleated amœba would thus behave like cells treated with dinitrophenol, which interrupts synthesis by blocking the coupling between oxidation and phosphorylation. Work is now in progress to find out if the nucleus, and in particular the nucleolus, takes any part in the synthesis of the coenzymes necessary for this coupling. Our results, added to those of Mazia and Hirshfield, give us a better understanding of the 'control' of syntheses by the nucleus.

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[1] Brachet, J., *Experientia*, **5**, 294 (1950).
[2] Clark, A. M., *Austral. J. Exp. Biol. Med. Sci.*, **20**, 241 (1942).
[3] Holter, H., and Zeuthen, E., *C.R. Trav. Lab. Carlsberg*, **26**, 277 (1946).
[4] Andresen, N., and Holter, H., *Science*, **110**, 114 (1949).
[5] Maria, D., and Hirshfield, H. I., *Science*, **112**, 297 (1950).

Ergothioneine in the Seminal Vesicle Secretion

Protein-free extracts from the secretory fluid of the seminal vesicles exhibit a marked reducing property towards potassium permanganate and Folin's phosphotungstic reagent in the cold, and towards 2,6-dichlorophenol-indophenol in acid solution. Boar vesicular secretion, which is available in large quantities, was chosen as a convenient source for the isolation of the reducing material. In the course of purification, it was found that the reducing power went parallel with two other chemical properties of the boar vesicular secretion, namely, a strongly