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RESOLUTION OF CHLORPHENIRAMINE AND THE PHARMACOLOGICAL PROPERTIES OF ITS ISOMERS

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CHLORPHENIRAMINE maleate ('Piriton', Allen and Hanburys, Ltd.) [3-(*p*-chlorophenyl)-3-(2′-pyridyl)-NN-dimethyl propylamine maleate][1,2] is a potent antihistamine[3]. We wish to report its resolution, and the physical constants, and the pharmacological properties of its optical isomers.

Chlorpheniramine proved somewhat difficult to resolve since it forms readily crystallizable salts with only a few acids. Initial attempts to form crystalline salts from fifteen optically active acids were not successful; however, after standing for one year, a solution of the di-*p*-toluoyl-(−)-tartrate[4,5] in aqueous ethanol deposited a few crystals. These were used to seed a freshly prepared solution giving (−)-chlorpheniramine-di-*p*-toluoyl-(−)-tartrate, which, after recrystallization to constant rotation, had melting point 135–136° C., $[\alpha]_D^{21} + 57{\cdot}4°$ (*c.* 1·1 in ethanol). This gave (−)-chlorpheniramine [$[\alpha]_D^{21{\cdot}5} - 31{\cdot}6°$ (*c.* 1·8 in ethanol)], which was converted to the hydrogen maleate [melting point 114–115° C., $[\alpha]_D^{27} - 23{\cdot}7°$ (*c.* 1·2 in water)].

The corresponding antipodes were obtained from the di-*p*-toluoyl-(+)-tartrate [melting point 135–36°, $[\alpha]_D^{20} - 57{\cdot}8°$ (*c.* 1·7 in ethanol)], which gave (+)-chlorpheniramine [$[\alpha]_D^{24} + 31{\cdot}6°$ (*c.* 1·3 in ethanol)], which was converted to the hydrogen maleate [melting point 114° C., $[\alpha]_D^{20} + 23{\cdot}1°$ (*c.* 2·0 in water)].

The anti-histamine activity of each isomer and the racemate was assessed by an *in vitro* method. The antagonistic effect of each drug on the contractions induced by histamine of the isolated ileum of the guinea pig was determined following the method of Schild[6]. The results expressed as pA_2 values (2 min. drug-tissue contact) are shown in Table 1.

The (+) isomer exhibited approximately twice the anti-histamine activity of the racemate, while the (−) isomer showed only about half the activity of the racemate. Further studies in which the pA_2 values of the drugs were assessed after 4 and 8 min. contact with the tissue showed that the duration of the anti-histamine action of neither isomer differed from that of the racemate.

Table 1

Compound	Anti-histamine activity pA_2 value (2 min. drug–tissue contact)
(+)-Chlorpheniramine maleate	8·47
(±)-Chlorpheniramine maleate	8·10
(−)-Chlorpheniramine maleate	7·81

Table 2

Compound	Acute intraperitoneal toxicity in mice *LD*50 mgm./kgm. ± standard error
(+)-Chlorpheniramine maleate	175·4 ± 9·7
(±)-Chlorpheniramine maleate	187·9 ± 6·7
(−)-Chlorpheniramine maleate	193·6 ± 7·5

The acute intraperitoneal toxicity of the racemate and isomers was studied in mice. Low doses of both racemate and isomers showed no observable reactions; however, high doses produced clonic convulsions followed by depression and death. Mortality was observed for seven days after administration of the drug. These results, expressed as the *LD*50 value in mgm./kgm. for each anti-histamine, are shown in Table 2. It is apparent that the acute toxicity of each isomer does not differ significantly from that of the racemate.

Sedation is one of the more common side-effects of many of the anti-histamine drugs in clinical use; it was therefore thought worth while to determine whether the racemate or either isomer caused any depression of the central nervous system. This was achieved by examining the compounds for their effect in modifying the narcotic activity of intraperitoneal injections of pentobarbitone sodium in mice. Narcotic activity was estimated using the rotating drum method of Collier, Hall and Fieller[7]. These results are summarized in Table 3, and show that neither of the isomers nor the racemate potentiates the action of the barbiturate. In fact, all three compounds, when injected intraperitoneally at a dosage of 100 mgm./kgm. at the same time as the pentobarbitone sodium, significantly reduce the duration of the narcosis without affecting the onset.

It has been well established that many anti-histamines have a local anæsthetic action, a property that contributes to the beneficial effects of topical application of these agents in painful allergic or other dermatological conditions. In this study the local anæsthetic activity of the isomers and the racemate were determined in guinea pigs using the intradermal injection method of Somers and Edge[8]. Racemic chlorpheniramine maleate showed similar local anæsthetic activity to that of procaine hydro-

Table 3

Compound	No. of mice	Dose (mgm./kgm. intraperitoneally)	Narcosis in mice				
			Pentobarb. sod. (mgm./kgm. intraperitoneally)	Onset (min. ± stand. error)	*P*	Duration (min. ± stand. error)	*P*
Saline	10	—	30	3·7 ± 0·5	—	16·1 ± 0·9	—
(+)-Chlorpheniramine maleate	10	100	30	3·7 ± 0·6	> 0·9	12·8 ± 1·0	< 0·05
(±)-Chlorpheniramine maleate	10	100	30	4·8 ± 1·0	> 0·3	11·6 ± 1·3	< 0·01
(−)-Chlorpheniramine maleate	10	100	30	3·8 ± 0·7	> 0·8	11·3 ± 1·6	< 0·02

chloride. The activity of the (−) isomer was approximately one and a half times, while the (+) isomer was only half that of the racemate.

It has been reported[9] that continued local use of anti-histamines may cause skin sensitization reactions. In view of the combination of potent anti-histaminic and local anæsthetic activity of chlorpheniramine maleate and its isomers, and their possible use in topical application, it was considered important to determine whether these compounds produced skin sensitivity. Formulated preparations of the racemate and the two isomers were therefore examined for their possible irritant action upon shaved rabbit skin. Each preparation contained 2 per cent of anti-histamine and was applied twice daily for two weeks. There was no evidence of any degree of skin sensitization during this time with any of the preparations. After treatment had stopped, the rabbits were exposed to sunlight and daylight for a further two weeks; at the end of this time there was no evidence of any photosensitivity reaction in the treated areas of skin.

The experiments performed indicate that the pharmacological activities of both the (+) and the (−) isomers of chlorpheniramine maleate are qualitatively similar to those of the racemate. The (+) isomer is more active than the racemate as an anti-histaminic drug, but is less active as a local anæsthetic; the reverse is true with the (−) isomer. In view of this quantitative difference in activity, it is interesting to note that the acute intraperitoneal toxicities of the compounds in mice are similar. It is proposed to compare the possible anti-tussive action of each of these isomers with that of the racemate.

While this communication was being written, the results of some pharmacological investigations on the optical isomers of chlorpheniramine were reported by Roth and Govier[10], but no details of the resolution or physical constants of the isomers were given. Anti-histamine and central nervous system activity and toxicity were investigated; our results are in agreement with theirs with the exception of the anti-histaminic activity of the (−) isomer, which was reported to be only 0·01 of that of the racemate.

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MORPHOGENETIC RESPONSE OF *CITRUS* OVULES TO GROWTH ADJUVANTS IN CULTURE

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OUR understanding of the growth processes of ovules and their individual parts, namely, the integuments, nucellus, endosperm and embryo from inception to the adult stage, is incomplete. A study of the growth and differentiation of 'entire' ovules (pre- and post-pollination stages) using tissue-culture technique as a tool is of great use in unravelling the physiological and morphological changes that occur within them following pollination and fertilization. Apart from a few cursory reports, ovule culture is yet an unexplored field. White[1] and LaRue[2] secured a callus growth on the ovules of *Antirrhinum* sown in White's medium. The ovules of *Erythronium americanum* showed a four-fold increase in size and an irregular growth of their surface. With orchids, Withner[3] found ovule culture to be quite promising for shortening the period elapsing between pollination and maturation of the seed, and for hastening the production of seedlings. Recently, Nirmala Maheshwari[4] has reported that poppy ovules cultured at the 2-celled stage of the embryo on Nitsch's basic medium containing a mixture of vitamins, glycine, kinetin (0·4 p.p.m.) and indoleacetic acid (5 p.p.m.) grew to mature seeds capable of germination *in situ*.

From relevant literature, it would appear that no culture work has so far been done on ovules possessing adventive embryos. The present investigation was undertaken with the view of exploring the nature of response of ovules that are naturally polyembryonate to such growth supplements as coconut milk, tomato juice and malt extract.

Citrus microcarpa Bunge was chosen as the ovule source. The young fruits were surface-sterilized and the ovules dissected out. Ovules, in which nucellar embryos had been just initiated, were inoculated on White's medium which was used as control[5]. 3 and 5 per cent sucrose were also tried as carbon source and the growth adjuvants used were Seitz-filtered.