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The Effect of Alkyl Substitution in Drugs—IV. Pharmacological Properties of Tropinyl 2-Methylbenzhydryl Ether Hydrobromide (BS 6825)

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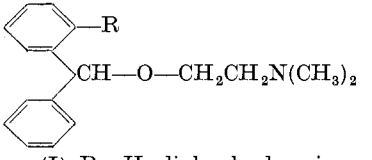
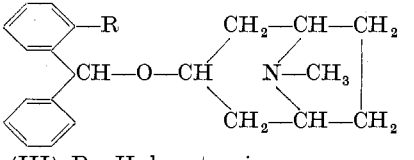
In the first article of this series[1] we reported on the pharmacological properties of a number of alkyl-substituted β-dimethylaminoethyl benzhydryl ethers. One of these, the 2-methyl derivative of diphenhydramine (I), the well-known antihistaminic, showed less antihistamine activity and markedly increased anti-acetylcholine action and is now extensively used as a therapeutic agent in Parkinsonism and in various other neurological and psychic disorders, under the name of orphenadrine (II).* In view of the fact that the benzhydrylether of tropine, benztropine (III),† is a much more potent drug than its dimethylaminoethyl congener and also shows marked anti-Parkinson activity, we decided to investigate whether introduction of a methyl group into the benztropine molecule would further enhance its anticholinergic activity and possibly reduce its strong antihistamine action. The compound envisaged (IV, BS 6825‡) was obtained by etherification of 2-methylbenzhydrol with tropine. It was isolated as the hydrobromide, m.p. 223–224°, and subjected to various pharmacological tests, along with benztropine itself.



(I) R = H, diphenhydramine
(II) R = CH_3, orphenadrine



(III) R = H, benztropine
(IV) R = CH_3, BS 6825

* Disipal ®; Mephenamine ®; Norflex ®; Brocadisipal ®.
† Cogentine ®.
‡ Patents applied for.

While this paper was being prepared, Farquharson and Johnston published their observations on the antagonism of tropine derivatives[2] on Tremorine effects. Their investigation covered, among other compounds, (I), (II) and (III), and the hydrochloride salt of (IV) (we used the hydrobromide). As the results of the investigation differ from ours on several points, we have discussed them under the respective sub-headings of the experimental part.

Experimental

Synthesis

Tropinyl 2-methylbenzhydryl ether hydrobromide. Tropine (155·1 g, 1·1 mole) was added to 2-methylbenzhydrol (198 g, 1·0 mole) and the mixture heated to 50–60° to form a homogeneous melt. Subsequent addition of *p*-toluenesulphonic acid (197·8 g, 1·15 mole) caused the temperature to rise to 90–100°, after which the mixture was heated for 4 to 5 h *in vacuo* at 130–150°. After cooling, the mixture was treated with a mixture of dilute sodium hydroxide solution and ether. The ethereal layer was separated and extracted with dilute hydrobromic acid solution; the hydrobromide separated as a rapidly solidifying oil. Isolation and crystallization of the product from acetone or a mixture of acetone and ether yielded 300 g (75 per cent) of tropinyl 2-methylbenzhydryl ether hydrobromide, m.p. 223–224°C.

Anal. Calcd. for $C_{22}H_{28}BrNO$: C, 65·67; H, 7·02. Found: C, 65·8; H, 7·3.

The substance is readily soluble in ethanol and warm acetone. Its solubility in cold water is 1 in 4000 and at 40° more than 1 in 200.

Pharmacology

Toxicity

Benztropine and BS 6825 were dissolved in water and injected, either subcutaneously or intravenously, into groups of 5 albino mice in volumes of 0·01 ml/g body weight. We used male and female mice from our own strain, 15–20 g in weight. The doses used increased logarithmically; they were plotted (in mg/kg) against mortality (per cent, recorded after 24 h). The LD_{50} was estimated graphically.

Table I. Acute LD_{50} (mg/kg in mice)

Mode of administration	Benztropine	BS 6825
s.c.	60 (55)[a]	55 (43)[a]
i.v.	25	25

[a] Farquharson and Johnston[2].

The toxic phenomena were about the same for both compounds: mydriasis, convulsions, co-ordination disturbances, gasping, followed by death through respiratory arrest. The slightly different values found by Farquharson and Johnston may be due to the different strain of mice used.

Oral toxicity in rats (TNO strain) was estimated, 6 doses of BS 6825 being administered to groups of 8 animals (4 ♂ and 4 ♀, 75–135 g in weight). Calculations according to Litchfield–Wilcoxon yielded an LD_{50} of 400 mg/kg (380–420).

Preliminary investigations into the subchronic toxicity of BS 6825 showed that a dose of 60 mg/kg administered daily to each of 6 rats (3 ♂ and 3 ♀), over a period of 23 weeks, only slightly impaired the growth as compared with control animals. No hematological, hepatic or renal abnormalities were found.

Spasmolytic Effect

Spasmolytic activity was measured on the isolated guinea pig ileum. The piece of intestine was suspended in Tyrode solution in a 50-ml vessel and stimulated with a dose of 1–2μg acetylcholine or histamine or with 5 mg of $BaCl_2$.

The contraction recorded was about 80 per cent of the maximum possible. After 5 min, BS 6825, in Tyrode solution, was added and the dose that would cause a 45–55 per cent reduction of the contraction was sought. On the same piece of intestine, but only if repeated washings with Tyrode solution had restored the tissue so that the same height of contraction was reached with the same dose of spasmogen, the equipotent dose of atropine or benztropine was estimated. Results are listed in Table II.

Our results clearly indicate an increased anti-acetylcholine activity of the *ortho*-substituted compound as compared with

Table II. Spasmolytic activity of BS 6825, compared with benztropine and atropine on a molecular basis

	Benztropine	Atropine	BS 6825 $\bar{x} \pm \sigma\sqrt{n}$[a]	n[a]
Anti-acetylcholine activity	1		4·3 ± 0·3	10
		1	4·3 ± 0·6	13
Anti-histamine activity	1		0·1 ± 0·008	8
Anti-$BaCl_2$ activity	1		1·6 ± 0·15	7

[a] n = number of experiments. $\bar{x}$ = average. σ = standard deviation.

benztropine itself. This, however, is completely at variance with the results of Farquharson and Johnston,[2] who report an activity of the methyl derivative which is only 60 per cent of that of the non-substituted compound. In our opinion the fact that they used a slightly different salt (hydrochloride as against hydrobromide) could hardly furnish an adequate explanation, while the different technique employed—Farquharson used a superfusion technique (Adam *et al.*[3])—would not account for such divergences. Moreover, the values obtained by Farquharson for orphenadrine HCl and diphenhydramine HCl—0·2 and < 0·01 (atropine = 1·0) resp.—differ considerably from ours [0·035 and 0·01 (atropine = 1·0)] and not consistently in the same direction.

Atropine-like and Antihistamine Effects in vivo

The antagonistic effect of benztropine and BS 6825 on the hypotension and the increased intestinal tonus induced by stimuation of the peripheral stump of the right vagus nerve, or by intravenous injection of carbachol (1 μg/kg) or histamine diHCl (1 μg/kg), was studied in cats narcotized with chloralose. A mercury manometer connected with the carotid artery was used to register the blood pressure, while the intestinal tonus was recorded by way of a duodenum balloon and a water manometer. Geometrically progressing doses of 1–2–4–8 μg etc. were given to each of three cats for each substance tested. Table III, therefore, indicates the range within which the effects mentioned were observed.

Table III. Anti-acetylcholine and antihistamine effect *in vivo* (ED in μg/kg)

	⩾50% inhibition of the hypotension by			⩾50% inhibition of the duodenum tonus by	
	Vagus stimulation	Carbachol i.v.	Histamine	Vagus stimulation	Carbachol i.v.
Benztropine	128–256	32–64	32	512	32–64
BS 6825	128	32–64	512	512	32–64

Histamine Micro-shock

Groups of 10 guinea pigs were exposed to an aerosol of a solution of histamine diHCl in glycerin and water (0·02:10:90). In the absence of an antagonist this led to severe dyspnoea within 0·5 to 1 min. Animals which were still without any symptoms, even without slight hyperpnoea, after 3 min, were considered protected. One hour before exposure, drugs were administered orally in 1 ml of a tragacanth mucilage. With BS 6825, 50 mg/kg protected 4 out of 10 guinea pigs; 75 mg/kg protected 6 out of 10 animals. In the case of benztropine, 5 mg/kg protected all 10 animals of a group, while 2·5 mg/kg protected 6 out of 10. This shows that BS 6825 *in vivo* as well as *in vitro* is a much weaker antihistaminic than benztropine.

Effect on Salivation

Using a method of Brown and Quinton[4] we found that salivary secretion, induced by subcutaneous administration of pilocarpine, was inhibited up to 50 per cent after 100–200 μg/kg of benztropine or BS 6825.

Mydriatic Activity

This was determined on the mouse pupil by the method of Pulewka,[5] the dilatation being measured at regular intervals after subcutaneous injection of atropine sulphate, benztropine or BS 6825. The P_A/P_B quotients were compared, P_A representing the largest diameter in each animal at a certain dose, and P_B the size of the pupil of the same animal before administration. Both benztropine and BS 6825 showed mydriatic activity of about one-fifth that of atropine sulphate. Farquharson used the method

of Ing *et al.*[6] by which the pupil diameter is measured only once (30 min after intraperitoneal administration) and found benztropine and tropinyl 2-methylbenzhydrylether to have 0·1 and 0·03 the activity of atropine respectively. Applied by us, the method of Ing yielded the same results as that of Pulewka, the tropinyl 2-methylbenzhydryl ether showing a consistent activity of 0·27 that of atropine. As Farquharson reported a mydriatic activity of 0·02 of that of atropine for orphenadrine HCl, in agreement with our experiments, the discrepancies are not readily explicable.

Effect on the Central Nervous System

Compulsive circling. According to Diamant[7] an antagonism against acetylcholine effects on the central nervous system can be demonstrated by means of compulsive circling induced in guinea pigs.

For this purpose eserine solution (0·3–0·4 mg/kg, 0·2 mg/ml) was injected into one of the *A. carotis* communes under local anaesthesia, which caused the guinea pigs to turn their heads contralaterally, occasionally followed by compulsive circling in the same direction. We used 75 guinea pigs and found that if benztropine (4·5–5 mg/kg) was administered subcutaneously 30 min before the eserine injection the effect failed to occur in 15 out of 30 animals; only 0·5–0·6 mg/kg of BS 6825 was required to obtain this result in 8 out of 16 animals. This shows that *ortho*-methyl substitution in benztropine results in an increased central anticholinergic effect.

Action against morphine mania in cats. In order to discover possible tranquillizing and/or anti-emetic activity, we subjected 4 cats to one of our routine tests to determine antagonism against the effect of a subcutaneous injection of morphine HCl 5 mg/kg. Neither benztropine nor BS 6825, not even in toxic doses of 5 mg/kg, could antagonize the vomiting and mania caused by the morphine.

Antagonism against Tremorine effect. Tremorine ® (1,4-dipyrrolidinobutyn-2) induces, in mice for instance, considerable tremor over the entire body, accompanied by salivation, lacrimation, diarrhoea and increased micturition; anti-Parkinson drugs inhibit these symptoms.[8] Benztropine or BS 6825 was administered

intraperitoneally 15 min before subcutaneous injection of Tremorine (20 mg/kg). The effect of the two compounds was the same: 3 mg/kg i.p. protected at least 50 per cent of the mice treated with Tremorine against the occurrence of the tremor for at least 2 h.

Farquharson and Johnston used different methods to determine this antagonism: the antagonist was administered either subcutaneously (30 min before) or orally (60 min before) subcutaneous administration of 30 mg/kg Tremorine. According to the former technique—which most resembles the one used by us—the ED_{50}, after 2 h, of benztropine was 1·2 mg/kg and of tropinyl 2-methylbenzhydrylether 1·5 mg/kg—results which are in close agreement with ours.

Antagonism against harmine tremor. Zetler[9] established that anti-Parkinson drugs inhibit the occurrence of tremor in mice after subcutaneous administration of 7 mg/kg of harmine. Fifty per cent of the mice were protected against the harmine tremor by 25–30 mg/kg benztropine or 15 mg/kg BS 6825 when the compound was injected intraperitoneally simultaneously with harmine HCl. The activity of the *ortho*-methyl compound is therefore twice as great. The active concentration of the two drugs falls in the range of the values found by Zetler for customary anti-Parkinson drugs.

Prolongation of the hexobarbital narcosis. Three groups of 18 mice each were used, the first receiving 25 mg/kg BS 6825 subcutaneously, the second 25 mg/kg benztropine subcutaneously, and the third physiological saline. After 30 min all animals received a 75 mg/kg dose of hexobarbital sodium solution (7·5 mg/ml; aq. dest.) intraperitoneally. The following sleeping times were recorded (in minutes):

A. BS 6825: 15, 16, 18, 20, 23, 28, 30, 30, 32, 32, 33, 40, 43, 60, 90, 90, 300, 305.
B. Benztropine: 15, 20, 25, 37, 46, 60, 65, 65, 72, 75, 85, 115, 120, 135, 208, 240, > 360, > 360.
C. Physiological saline: 5, 8, 8, 10, 12, 15, 19, 20, 21, 22, 24, 25, 25, 26, 50, 170, 200, 210.

Median, with confidence limits $\alpha < 0{\cdot}05$[10]: BS 6825: 31 (23–43); benztropine: 68·5 (46–120); physiological saline: 20·5 (12–25).

As the values obtained in this type of test were not normally

distributed (Rümke and Bout[11]), we used the modified Wilcoxon two-sample test according to Mann and Whitney[12] (see also van Eeden and Rümke[13]). Comparison of groups C and A showed that BS 6825 increases the sleeping time as compared with the control group ($P < 0{\cdot}05$). Comparison of groups A and B revealed that benztropine increases the sleeping time significantly more than BS 6825 ($P < 0{\cdot}02$).

Influence on the conditional avoidance reflex in rats. An effect on the conditional reflex is considered an indication that the drug in question has tranquillizing properties if at the same time the unconditional reflex remains unaffected. We used the method of Cook *et al.*[14] modified according to van Proosdij-Hartzema[15] with pole-climbing rats and found that the conditional avoidance reflex was 80 per cent inhibited after intraperitoneal administration of BS 6825 (20 mg/kg); the unconditional reflex (after application of electric shocks) remained intact. The same amount of benztropine caused a 40 per cent inhibition.

Discussion

Although we are unable to explain the discrepancies between our results and those found by Farquharson and Johnston during some of their experiments, we feel justified in stating that introduction of an *ortho*-methyl group in benztropine results in a change in anti-acetylcholine and antihistamine activities which is largely parallel to that found when diphenhydramine HCl and orphenadrine HCl are compared.

Not only does BS 6825 show greater activity against acetylcholine spasm of the isolated guinea-pig ileum, but it is also much more effective against compulsive circling in guinea pigs after eserine administration, which is considered an indication for central anticholinergic activity. Whether the greater inhibition of the harmine tremor shown by BS 6825 may be due to the same or related mechanisms is not certain.

Furthermore, the antihistamine activity is greatly reduced by the introduction of the *ortho*-methyl group, as appears from the weaker action of BS 6825 on the isolated guinea-pig ileum against histamine, against hypotension induced by histamine and against bronchoconstrictory action of histamine aerosol.

Whether the weaker prolonging effect of BS 6825 on the hexobarbital sleeping time is due to its weaker antihistamine activity or its absence of soporific effect (a side effect often encountered with benztropine and with many antihistaminics) is a subject for speculation.

With respect to various other pharmacological properties, such as inhibition of salivary secretion and effect on the pupil diameter, there is no difference between the substituted and the non-substituted compound.

Clinical Application

BS 6825 in a dose of 2–4 mg daily was clinically compared with orphenadrine in patients suffering from Parkinson-type symptoms during treatment with neuroleptics, and in those with various other psychic disorders. BS 6825 shows activity in these conditions but exhibits side effects, such as dry mouth and disturbances of accommodation.

Summary. BS 6825, or tropinyl 2-methylbenzhydryl ether hydrobromide, was synthesized in order to investigate the influence of a 2-methyl group on pharmacological activity. The substance proved to be a stronger anticholinergic and a less active antihistaminic than the non-substituted compound, benztropine. Toxicity and several other properties of BS 6825 were studied.

A preliminary clinical investigation has yielded favourable results in cases with Parkinson-type symptoms, in manic states and in neurotic depressions.

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