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Quaternary Ammonium Compounds—II. Antiacetylcholinesterase Activity and Charge Distribution in Aliphatic Quaternary Ammonium Compounds

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Introduction

Probably the most widely accepted theory relating to the way in which acetylcholinesterase unites with substrates is that of Nachmansohn.[1] The substrate, usually acetylcholine, is considered to unite with the enzyme at two points: the positively charged nitrogen atom with the anionic site and the acyl carbon of the ester group with the esteratic site. Antiacetylcholinesterases such as neostigmine and eserine are thought to unite with the same active sites but to differ from the substrates in being hydrolysed either not at all or only very slowly. However, many simple aliphatic quaternary ammonium compounds which contain no other polarized group possess antiacetylcholinesterase activity. It is considered that their positively charged nitrogen atom becomes associated with the anionic enzyme site and that either this in itself is sufficient to inhibit the enzyme or that the bulk of the attached quaternary ammonium ion prevents access to the esteratic site of the enzyme. From these considerations, it follows that there is a relation between the forces of adsorption of quaternary ammonium compounds onto acetylcholinesterase and the antiacetylcholinesterase activity of these compounds. The total adsorption force between cholinesterase and quaternary ammonium compounds is comprised of the following constituent forces.[2,3]

(*a*) Coulombic interaction between the positive charge of the quaternary ammonium group and the anionic site of the enzyme.

(*b*) Van der Waal's forces between the hydrocarbon moiety of the quaternary ammonium ion and the enzyme surface.

Since the positive charge in aliphatic onium compounds remains constant from one compound to the next, and hence the potential coulombic force remains constant, then it would appear that the antiacetylcholinesterase activities of quaternary ammonium compounds should increase with any addition to the hydrocarbon moiety, i.e. an increase in the van der Waal's forces. This effect has been demonstrated with the series of trimethylalkylammonium compounds[2,4] where it was found that as the homologous series was ascended so the compounds became more active as inhibitors of acetylcholinesterase.

However, other factors must be involved since it has been shown that an increase in the hydrocarbon moiety of a series of quaternary ammonium compounds does not always increase the activity of the compounds. Bergmann and Shimoni[4] have determined the inhibition of acetylcholinesterase obtained with the series of quaternary ammonium salts of the type R_4N^+ where R was methyl, ethyl, *n*-propyl and *n*-butyl in turn. With these symmetrically substituted ions maximum activity was obtained with the tetra-*n*-propylammonium ion. The lower effectiveness of tetra-*n*-butylammonium was ascribed to the limited space available at the active surface, rendering it more difficult for this ion to approach the anionic site closely.

A similar type of result was obtained by Blaschko and coworkers[5] who examined the inhibition of acetylcholinesterase by homologues of dimethylcarbamic ester of 3-hydroxyphenyltrimethylammonium bromide (neostigmine bromide) in which the methyl groups on the quaternized nitrogen atom were replaced in turn by ethyl groups. It was found that the most active compound was the diethylmethylamino homologue; the triethyl compound was less active than neostigmine bromide.

A further study which demonstrated the importance of alkyl groups on the antiacetylcholinesterase action of onium compounds was that of Wilson[6] who examined the inhibition obtained with a series of methylated ammonium compounds and also with a series of methylated ethanolamine compounds all of which were cationic at pH 7. He found, in both series, that the activity increased with the introduction of methyl groups except in the case of the

last methyl group. In both series the tertiary amino conjugate acid was as active as the corresponding quaternary ammonium salt. This was explained by a consideration of the tetrahedral structure of the ammonium group which made it impossible for the protein to come into close contact with all four alkyl groups, unless the protein was able to fold itself about the ion so as to engulf it. Apparently such a re-orientation of the protein did not occur, or if it did the attending increase in free energy which such a process would imply just offset the decrease in energy due to extra binding of the quaternary and the cationic tertiary inhibitors, which would appear to be highly improbable.

It is obvious from the evidence presented that factors other than Coulombic and van der Waal's forces are involved in the binding of quaternary ammonium compounds to acetylcholinesterase because indiscriminate addition of methylene groups to a quaternary ammonium compound does not necessarily increase its activity. It is possible to interpret these results in terms of the structure of the quaternary ammonium ion by postulating that the major electrostatic attraction between the quaternary ammonium group and the anionic site of the enzyme is between the fractional positive charge on the α carbon atoms of the onium ion and the enzyme, rather than between the charge on the nitrogen atom and the enzyme surface. In arriving at this postulate, two properties of the quaternary ammonium group have been considered, (*a*) the stereochemistry and (*b*) the distribution of the unit positive charge among the nitrogen and the four α carbon atoms. The groups bonded to a nitrogen atom in the quaternized state are arranged in a tetrahedral configuration.[7] Pauling[8] has calculated that the unit positive charge of the ammonium ion is not located exclusively on the nitrogen atom as was originally assumed but is distributed equally among the four hydrogen atoms and the nitrogen atom. A similar situation occurs with quaternary ammonium ions such as tetraalkylammonium salts.[9] In such compounds the unit positive charge is distributed, to the first approximation, among the four α carbon atoms and the nitrogen atom. Examination of models of such alkyl quaternary ammonium compounds shows that the α carbon atoms are able to come much nearer to a surface than the nitrogen atom. Therefore, remembering that the force between charged particles diminishes

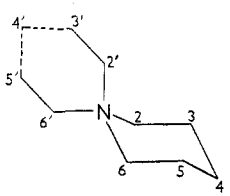
as the square of the distance between them, it seems probable that the affinity of these inhibitors for the enzyme is due to attraction between the anionic site of the enzyme and the δ^+ charge on the α carbon atoms with a smaller contribution from the more distant δ^+ charge on the quaternary nitrogen atom. If this is the case, the pattern of the results obtained with the homologues of neostigmine bromide is explicable. The replacement of one methyl group by an ethyl group on the quaternary ammonium nitrogen atom increased the activity of the molecule by increasing both the van der Waal's forces and the surface activity of the inhibitor. The introduction of a second ethyl group had a similar effect and consequently increased the activity of the molecule. However, the introduction of a third ethyl group decreased the overall binding between the inhibitor and enzyme to below the level of the trimethylammonium homologue. This suggests that the β carbon atoms prevent the α carbon atoms from coming into close contact with the anionic site of the enzyme, thus reducing the electrostatic forces of attachment. Up to the present there are no other experimental results to corroborate the postulate. The inhibition experiments which have been carried out on simple quaternary ammonium compounds such as tetramethylammonium and its homologues have suffered from the disadvantage that, because of free rotation of the carbon to carbon bonds of the alkyl groups, the availability of the α carbon atoms could not be determined. A second factor which complicates the interpretation of the results obtained with these ions is the rise in surface activity of amphipathic molecules as the lyophobic to lyophilic ratio is increased. A result of this effect will be that as a homologous series of compounds, such as the trimethylalkylammonium series, is ascended so the antiacetylcholinesterase activity will rise because of (*a*) the increased forces of attraction between enzyme and inhibitor and (*b*) the increased concentration of onium ions at the interface of the water and enzyme due to the effect of the water on the ions.

In order to obtain evidence to support the charge distribution postulate, a series of stereospecific quaternary ammonium compounds in which the only difference between the compounds is the availability of the α carbon atoms has been prepared and tested for antiacetylcholinesterase activity. Since all the quaternary

ammonium compounds prepared have the same or similar molecular weights and have similar structures, then the surface activity effect should be practically the same for each compound. The following spiran quaternary ammonium compounds have been prepared and examined for antiacetylcholinesterase activity: 1,1′-spirobipiperidinium bromide; *cis*-2,6-dimethyl-1,1′-spirobipiperidinium bromide; *trans*-2,6-dimethyl-1,1′-spirobipiperidinium bromide; 2,6-bimethylene-1,1′-spirobipiperidinium bromide. In part I of this series[10] it was shown that spiran formation did not have any special effect on the antiacetylcholinesterase activity of this type of quaternary ammonium compound.

Stereochemistry of Spiran Quaternary Ammonium Compounds

The 1,1′-spirobipiperidinium ion consists of two piperidine rings linked by a common nitrogen atom (I). Assuming that the two piperidine rings are in the chair conformation, then an examination of molecular models of this ion shows that carbon atoms 2, 6 and 6′ are in a plane. The nitrogen atom is in between these carbon atoms and above the plane, while the 2′ carbon atom is tucked away on the top side of the ion. This ion can therefore come into contact with the enzyme in such a manner that three α carbon atoms, 2, 6 and 6′, are all directed towards the enzyme surface. By substituting methyl groups for the hydrogen atoms on the carbon atoms 2 and 6, it is possible to interfere with the plane formed by carbon atoms 2, 6 and 6′ and thus with the number of α carbon atoms which are available for binding to the enzyme. Carbon atoms 2, 2′ and 6′ also form a second triad of α carbon atoms in a plane. Since the molecule is symmetrical then these two planes are identical in all respects. The two planes will be referred to as plane A (2, 6, 6′) and plane B (2, 2′, 6′) in the subsequent discussion.

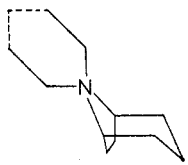


(I)

2,6-Dimethyl-1,1′-spirobipiperidinium ion. If it is accepted that the piperidine ring exists in the chair form, and there is much evidence for this (see references[11]), then there are three possible conformations for 2,6-dimethyl-1,1′-spirobipiperidinium: (II), (III) and (IV). Structures (II) and (III) represent the two possible conformations of the *cis* isomer. It is generally the case that substitutents in cyclic systems are more stable in the equatorial conformation than in the axial conformation but in the case of *cis*-2,6-dimethyl-1,1′-spirobipiperidinium bromide, because of interaction between 6-methyl *e* and hydrogen atoms on the carbon atoms 3′ and 5′, it is considered that the more stable conformation will be 2-methyl *a*, 6-methyl *a* (III). It would be anticipated therefore that *cis*-2,6-dimethyl-1,1′-spirobipiperidinium bromide should be a more powerful inhibitor of acetylcholinesterase than 1,1′-spirobipiperidinium bromide because the introduction of the methyl groups will increase the van der Waal's forces but not reduce the availability of the α carbon atoms in plane B. The conformation of the *trans* isomer (IV) of 2,6-dimethyl-1,1′-spirobipiperidinium bromide is such that one methyl group is equatorial and one is axial and therefore theoretically two conformations can exist *viz*: 2-methyl *a*, 6-methyl *e* and 2-methyl *e*, 6-methyl *a*. It is suggested that the second conformation is the favoured one because of the possibility of interaction between an equatorial group on the 6 carbon and the hydrogen atoms on carbon atoms 3′ and 5′. If the conformation is 2-methyl *e*, 6-methyl *a* then both planes A and B are hindered and so only two of the α carbon atoms can come into contact with the anionic site of the enzyme. The Coulombic forces of attraction, therefore, should be less in the case of the *trans* isomer than in the case of the *cis* isomer while the potential van der Waal's forces are equal in the two compounds. The overall result should be that the *trans* isomer will be less active as an inhibitor of acetylcholinesterase than the *cis* isomer.

(II) (III) (IV)

2,6-Bimethylene-1,1'-spirobipiperidinium ion. The stereochemistry of this compound (V) is almost the same as the favoured *a, a* conformation of the *cis*-2,6-dimethyl-1,1'-spirobipiperidinium ion (III). In this case plane B is available and so the antiacetylcholinesterase activity should be similar to the *a, a* conformation of *cis*-2,6-dimethyl-1,1'-spirobipiperidinium bromide (III). The argument is the same if a boat conformation for tropane is considered.



(V)

Preparation of the Compounds

All the spiran quaternary ammonium compounds were prepared by condensing the corresponding substituted piperidine with 1,5-dibromopentane.[11] *Cis-* and *trans*-2,6-dimethylpiperidine were prepared by a sodium–butanol reduction of 2,6-dimethylpyridine followed by careful separation of the products. Marcuse and Wolffenstein[12] first reduced 2,6-dimethylpyridine by a sodium–methanol reduction and isolated two products, 2,6-lupetidine and 2,6-isolupetidine but they were unable to determine which was the *cis* isomer and which was the *trans*. Catalytic hydrogenation of 2,6-dimethylpyridine has been reported to give only lupetidine[13,14] and this has been assumed to be the *cis* isomer without evidence.[15,16] Hall has attempted to prepare *trans*-2,6-dimethylpiperidine by a number of methods but has failed to isolate it.[17] The two isomers have now been isolated from a sodium–butanol reduction of 2,6-dimethylpyridine by careful fractionation of the product followed by crystallization of the base hydrobromide obtained from the residues in the fractionating flask. Two products corresponding to lupetidine and *iso*lupetidine were obtained. Since the *cis* isomer is the meso form then only the *trans* isomer is capable of resolution. Lukes and Jizba[18] have prepared *cis-* and *trans*-1,2,6-trimethylpiperidine and partially resolved the *trans* isomer by means of the tartrate salt.

The two products obtained in the present work were converted to the *N*-methyl derivatives by the Eschweiler–Clarke reaction[19, 20] and the characters of the derivatives compared with those given by Lukes and Jizba. 2,6-Lupetidine is the *cis* isomer and 2,6-isolupetidine is the *trans* isomer. This is in accord with the fact that 2,6-isolupetidine is not formed at all by catalytic hydrogenation of 2,6-dimethylpyridine and only in small yield in a sodium–butanol reduction. Marcuse and Wolffenstein[12] reported a mixture of 30 per cent 2,6-isolupetidine and 70 per cent 2,6-lupetidine from a sodium–methanol reduction of 2,6-dimethylpyridine. No one has since been able to obtain yields of this order of 2,6-isolupetidine and it appears possible that the sample of 2,6-dimethylpyridine originally used was contaminated with other alkylpyridines, as it is only recently that a method has been discovered of obtaining 2,6-dimethylpyridine free from α and β picolines.[21]

Nortropane has been reported in the literature[22–25] and has now been prepared by reducing tropinone to tropane with a Wolff–Kishner reduction followed by demethylation with hypochlorous acid.[25]

Attempts to prepare 2,2,6-trimethyl-1,1′-spirobipiperidinium bromide by condensing 1,5-dibromopentane with 2,2,6-trimethylpiperidine were unsuccessful. In all cases elimination occurred with the formation of 2,2,6-trimethylpiperidinium bromide.

Experimental

Chemical

2,6-Dimethylpiperidine. 2,6-Dimethylpyridine (b.p. 142°; 103 g) was reduced by sodium in dry butanol to 2,6-dimethylpiperidine by the method of Pliml *et al.*[26] Yield 52 g; 45 per cent.

Isolation of cis *and* trans *isomers of 2,6-dimethylpiperidine.* A mixture of amine (63 g) and *p*-cymene (b.p. 177°; 15 ml) was fractionated at a reflux ratio of 40:1 through an 18-in. fractionating column packed with $\frac{1}{16}$-in. Dixon rings. A fraction of b.p. 127·7° at 768·8 mm was collected as the major component. When the temperature of the vapours began to rise the distillation was stopped. The residues from the distillation flask were extracted with hydrobromic acid (10 per cent), and the acidic

layer separated and evaporated to dryness under reduced pressure when a syrupy tar was obtained. This was dissolved in water (100 ml), the solution made alkaline with sodium hydroxide solution (50 ml; 20 per cent w/v) and the mixture steam distilled until 500 ml of distillate had been obtained. After acidifying the distillate with hydrobromic acid, and evaporating the solution to dryness under reduced pressure, the solid obtained was recrystallized from absolute ethanol; yield 1·6 g. A further crop of crystals (0·33 g) was obtained by the addition of solvent ether to the mother liquors. The filtrate was evaporated to dryness, the residue dissolved in water (10 ml) and the solution made alkaline with sodium hydroxide solution (10 ml; 20 per cent). The mixture was extracted with ether, the ethereal solution dried over anhydrous magnesium sulphate and then filtered. Dry hydrogen chloride gas was bubbled through the dry ethereal solution when amine hydrochloride was precipitated; this was filtered and dried: yield 0·43 g.

trans-*2,6-Dimethylpiperidine hydrobromide* m.p. 243–244° (isolupetidine hydrobromide m.p. 245°[12]).

Anal. Calcd. for $C_7H_{16}BrN$: Br, 41·2. Found: Br, 41·12. Yield of *trans*-2,6-dimethylpiperidine 1·44 g, 2·9 per cent.

trans-*2,6-Dimethylpiperidine hydrochloride* m.p. 231–232° (isolupetidine hydrochloride m.p. 232–234°[12]).

cis-*2,6-Dimethylpiperidine hydrobromide* m.p. 285° (lupetidine hydrobromide m.p. 285°[12]).

Anal. Calcd. for $C_7H_{16}BrN$: Br, 41·2. Found: Br, 41·18.

cis-*1,2,6-Trimethylpiperidine.* This was prepared by the Eschweiler–Clarke reaction using the method reported by Perrine,[20] b.p. 148–150°.

cis-*1,2,6-Trimethylpiperidine picrate* m.p. 224–225° (lit.[18] m.p. 224–225°).

cis-*1,2,6-Trimethylpiperidine hydrobromide* m.p. 268–269° (lit.[18] m.p. 268–269°).

Anal. Calcd. for $C_8H_{18}BrN$: Br, 38·4. Found: Br, 38·6.

trans-*1,2,6-Trimethylpiperidine.* This was prepared by condensing methyl iodide with *trans*-2,6-dimethylpiperidine.

trans-*1,2,6-Trimethylpiperidine picrate* m.p. 241–242° [lit.[18] 245 (d.)].

Tropane. Triethylene glycol (250 ml), sodium hydroxide (8 g),

tropinone (10 g) and hydrazine hydrate solution (10 ml, 85 per cent) were placed in a 500-ml round bottomed flask which was fitted with a reflux condenser and a side arm in which was placed a thermometer dipping below the surface of the liquid. The mixture was refluxed for 2 h and then distilled until the temperature of the mixture had risen to 185° when distillation was stopped and the mixture refluxed for a further 3 h. After allowing the mixture to cool, it was steam distilled until 250 ml of distillate was obtained. The first distillate was added to the steam distillate, the mixture acidified with concentrated hydrochloric acid (20 ml) and then evaporated to dryness under reduced pressure. The solid residue obtained was dissolved in the minimum quantity of water (15 ml), the solution made alkaline with sodium hydroxide solution (20 ml, 20 per cent w/v) and extracted with solvent ether. After drying the ethereal solution over anhydrous magnesium sulphate, it was filtered, the ether distilled and the residue fractionally distilled, and the fraction of b.p. 162° was collected. Yield of tropane, 8·54 g (97 per cent).

Tropane picrate, m.p. 281° (lit.[27] 280–281°).

Anal. Calcd. for $C_{14}H_{18}N_4O_7$: C, 47·45; H, 5·08. Found: C, 47·31; H, 5·1.

Nortropane. Tropane (5 g) was demethylated by the method of von Braun.[25] Yield 1·9 g.

Nortropane hydrochloride m.p. 284° (d.) [lit.,[22] 285° (d.)].

Anal. Calcd. for $C_7H_{14}ClN$: Cl, 24·3. Found: Cl, 24·2.

Spiran quaternary ammonium compounds. All the spiran compounds were prepared by condensing the appropriate amine

Table I. Spiran quaternary ammonium compounds prepared

Compound	m.p., °C	Calcd.	Found
cis-2,6-Dimethyl-1,1′-spiro-bipiperidinium bromide	254–255	C, 55·0 H, 9·16 Br, 30·5	C, 55·1 H, 9·2 Br, 30·3
trans-2,6-Dimethyl-1,1′-spiro-bipiperidinium bromide	263–264(d.)	C, 55·0 H, 9·16 Br, 30·5	C, 54·3 H, 9·25 Br, 30·8
2,6-Bimethylene-1,1′-spiro-bipiperidinium bromide	344(d.)	C, 55·4 H, 8·46 Br, 30·8	C, 55·4 H, 8·46 Br, 30·8

(1 mole), sodium hydroxide (1 mole) and 1,5-dibromopentane (1 mole) in ethanol. The products were worked up as previously described.[10] The compounds prepared are listed in Table I together with analytical data.

Antiacetylcholinesterase

The antiacetylcholinesterase activities of the compounds were determined by the Warburg method using the conditions given in Table II.

Table II. Antiacetylcholinesterase activities of spiran quaternary ammonium compounds. I_{50} in gram moles per litre. Erythrocyte stromata used as source of enzyme. Temperature 37°. Substrate concentrations 0·0033 M at pH 6·3 and 0·0166 M at pH 7·4

Compound[a]	pH 6·3	pH 7·4	No. of planes in which all α carbons are fully available for binding
1,1′-Spirobipiperidinium[10]	$1{\cdot}4 \times 10^{-2}$	$4{\cdot}9 \times 10^{-2}$	2
cis-2,6-Dimethyl-1,1′-spirobipiperidinium	$3{\cdot}33 \times 10^{-3}$	$7{\cdot}2 \times 10^{-3}$	1
trans-2,6-Dimethyl-1,1′-spirobipiperidinium	$2{\cdot}03 \times 10^{-2}$	$5{\cdot}06 \times 10^{-2}$	0
2,6-Bimethylene-1,1′-spirobipiperidinium	$2{\cdot}2 \times 10^{-3}$	$3{\cdot}2 \times 10^{-3}$	1

[a] All the compounds were used as the bromide.

Discussion

Table II indicates that the antiacetylcholinesterase activities of the spiran quaternary ammonium compounds are in the same order as was predicted assuming that the major contribution to the electrostatic binding is between the charge on the α carbon atoms and the enzyme. The influence of van der Waal's forces on the activities of *cis*-2,6-dimethyl-1,1′-spirobipiperidinium bromide and 2,6-bimethylene-1,1′-spirobipiperidinium bromide in which only one plane is available for binding outweighs the statistical advantage of 1,1′-spirobipiperidinium bromide in which two planes are available. An examination of models

indicates that each of the molecules prepared may be orientated towards a surface in such a manner that there is no difference in the availability of the nitrogen atom, and so it is difficult to explain the differences in activity if the charge on the nitrogen atom provides the major contribution to electrostatic binding. Also the differences in activity cannot be ascribed to differences in bulk, as was done by Bergmann[4] with the symmetrical tetra-alkylammonium compounds, because all the molecules are practically the same size. Finally, it is unlikely that the differences in activity are a result of different surface properties of the solutions of the compounds examined since all the quaternary ammonium ions had almost the same molecular weight and were of the same general type.

Summary. From an analysis of the stereochemistry and charge distribution of aliphatic quaternary ammonium compounds and the reported antiacetylcholinesterase activities of these compounds, it has been postulated that the major contribution to the electrostatic binding of these ions to acetylcholinesterase is between the α carbon atoms of the quaternary ammonium group and the anionic site of the enzyme. In order to obtain experimental evidence of this postulate, some quaternary ammonium spiran compounds of known configuration have been prepared and examined for antiacetylcholinesterase activity. The only difference between the compounds was the availability of the α carbon atoms for binding to an anionic site. The antiacetylcholinesterase activities obtained were in the order predicated by the postulate.

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References

[1] Nachmansohn, D. and Wilson, I. B. *Advanc. Enzymol.*, **12**, 259 (1951)
[2] Bergmann, F. *Disc. Faraday Soc.*, **20**, 126 (1955)
[3] Bernhard, S. A. *J. Amer. chem. Soc.*, **77**, 1966 (1955)
[4] Bergmann, F. and Shimoni, A. *Biochim. biophys. Acta*, **10**, 49 (1953)
[5] Blaschko, H., Bulbring, E. and Chou, T. C. *Brit. J. Pharmacol.* **4**, 29 (1949)
[6] Wilson, I. B. *J. biol. Chem.*, **197**, 215 (1952)
[7] Mills, W. H. and Warren, E. H. *J. chem. Soc.*, **127**, 2507 (1925)

[8] Pauling, L. *The Nature of the Chemical Bond*, p. 71. 1948. New York; Cornell University Press
[9] Taylor, D. B. *Pharmacol. Rev.*, **3**, 412 (1951)
[10] Thomas, J. This Journal, **3**, 45 (1961)
[11] Robinson, J. B. M.Sc. Thesis, (1958) Manchester
[12] Marcuse, A. and Wolffenstein, R. *Ber. dtsch. chem. Ges.*, **32**, 2528 (1899)
[13] Adkins, H. *J. Amer. chem. Soc.*, **56**, 2425 (1934)
[14] Overberger, C. G. *J. Amer. chem. Soc.*, **77**, 4101 (1955)
[15] Robinson, R. A. *J. org. Chem.*, **16**, 1911 (1951)
[16] Williams, A. R., Hidalgo, J. and Halverstadt, I. F. *J. Amer. pharm. Ass., Sci. Ed.*, **45**, 423 (1956)
[17] Hall, H. K. *J. Amer. chem. Soc.*, **79**, 5444 (1957)
[18] Lukes, R. and Jizba, J. *Chem. Listy*, **46**, 622 (1952)
[19] Eschweiler, W. *Ber. dtsch. chem. Ges.*, **38**, 880 (1905)
[20] Perrine, T. D., *J. org. Chem.*, **16**, 1303 (1951)
[21] Reithof, G. U.S. Patent 2,295,606
[22] Ladenburg, A. *Ber. dtsch. chem. Ges.*, **20**, 1647 (1887)
[23] Hess, K. *Ber. dtsch. chem. Ges.*, **51**, 1007 (1918)
[24] von Braun, J. *Ber. dtsch. chem. Ges.*, **44**, 1252 (1911)
[25] von Braun, J. and Rath, K. *Ber. dtsch. chem. Ges.*, **53B**, 601 (1920)
[26] Pliml, J., Knoblock, E. and Protiva, M. *Chem. Listy*, **46**, 758 (1952)
[27] Ladenburgh, A. *Ber. dtsch. chem. Ges.*, **16**, 1403 (1883)
[28] Gough, G. A. C. and King, H. *J. chem. Soc.*, 2444 (1928)