J. Pharm. Pharmac., 1970, **22**, 889–896 *Received April* 14, 1970

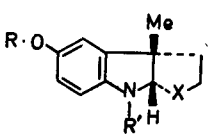
The synthesis and anti-acetylcholinesterase activities of (+)-physostigmine and (+)-physovenine*

F. J. DALE AND B. ROBINSON

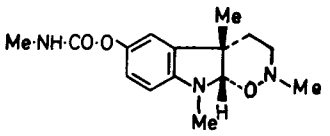
Department of Pharmacy, The University of Manchester, Manchester 13, *U.K.*

(+)-Physostigmine and (+)-physovenine have been synthesized. The anti-acetylcholinesterase activities of these two bases, which have been investigated *in vitro* using erythrocyte acetylcholinesterase, have been found to be much lower than the corresponding activities of the alkaloids (−)-physostigmine and (−)-physovenine. Possible reasons for these activity differences are discussed.

(−)-Physostigmine (eserine), the major alkaloid of *Physostigma venenosum* seeds (Calabar beans), which has recently (Hill & Newkome, 1969; Longmore & Robinson, 1969a, b; Newkome & Bhacca, 1969) been found to have the absolute configuration shown in I (R=Me·NH·CO, R′=Me, X=NMe), and a large number of synthetic analogues have been evaluated for anti-acetylcholinesterase activity (Long, 1963; Long & Evans, 1967; Stempel & Aeschlimann, 1956) and the chemical features essential for such activity have been established (Long, 1963; Long & Evans, 1967; Stempel & Aeschlimann, 1956). The structures and absolute configurations of the minor alkaloids of *Physostigma venenosum* seeds, (−)-physovenine, (−)-N_a-norphysostigmine, (−)-eseramine and (−)-geneserine have also recently been established to be as shown in I (R=Me·NH·CO, R′=Me, X=O; R=Me·NH·CO, R′=H, X=NMe; R=Me·NH·CO, R′=Me, X=N·CO·NH·Me) (Longmore & Robinson, 1969a, b; Robinson, 1968) and II (Longmore & Robinson, 1969a, b; Hootelé, 1969; Robinson & Moorcroft, 1970), respectively. Furthermore, *in vitro* studies using erythrocyte acetylcholinesterase have shown (Robinson & Robinson, 1968) that whereas the anti-acetylcholinesterase activities of (−)-physostigmine, (−)-N_a-norphysostigmine and



I

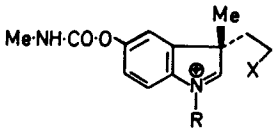


II

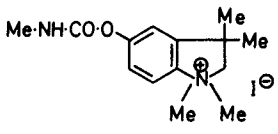
(−)-physovenine are approximately the same, (−)-eseramine and (−)-geneserine are devoid of anti-acetylcholinesterase activity under the same experimental conditions. These enzymological results, together with comparative data on the chemical behaviour of this series of alkaloids under conditions of varying pH, led to the suggestion (Robinson & Robinson, 1968) that the reactive species responsible for the anti-acetylcholinesterase activity of (−)-physostigmine is not the N_b-protonated species

* Alkaloids of *Physostigma venenosum*, Part X; for Part IX see Robinson & Moorcroft (1970).

(I; R=Me·NH·CO, R′=Me, X=$\overset{\oplus}{N}$H·Me) (modified to show the absolute configuration) as had previously (Wilson & Bergmann, 1950) been supposed, but the ring C-opened 3*H*-indolium cation III (R=Me, X=$\overset{\oplus}{N}H_2Me$) (showing the absolute configuration), the analogous cations (III; R=H, X=$\overset{\oplus}{N}H_2Me$ and R=Me, X=OH) being the active forms of (−)-N_a-norphysostigmine and (−)-physovenine, respectively. It was suggested (Robinson & Robinson, 1968) that the opening of the C-ring to give the 3*H*-indolium cations may occur at the acetylcholinesterase surface. The observation (see Table 1) that the anti-acetylcholinesterase activity of 1,1,3,3-tetramethyl-5-methylcarbamoyloxyindolinium iodide (IV) (Ahmed & Robinson, 1965) is far greater than that of (−)-physostigmine adds further support to this suggestion (see also discussion section).



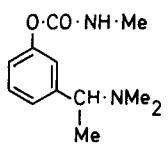
III



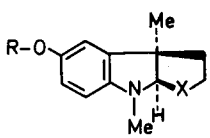
IV

A number of enantiomeric pairs of compounds have been assessed as cholinesterase inhibitors (Long, 1963): it has been found that the (−)-isomer of isomethadone (V) is thirty times more active than the (+)-isomer (Greig & Howell, 1948; Long, 1963), the (+)-isomers of active (−)-amino acids are far weaker inhibitors than the (−)-isomers (Bergmann, Wilson & Nachmansohn, 1950; Long, 1963) and it is the (−)-isomer of miotine (VI) which is the active isomer (Easson & Stedman, 1933; Long, 1963). As the absolute configurations of (−)-physostigmine and (−)-physovenine are established (see above), we have now synthesized (+)-physostigmine (VII, R=Me·NH·CO, X=NMe) and (+)-physovenine (VII; R=Me·NH·CO, X=O) and compared their anti-acetylcholinesterase activities with those of (−)-physostigmine and (−)-physovenine. The results of these studies will help in the further elucidation of the stereochemical requirements of the acetylcholinesterase active centres, which in some cases are similar to those of muscarinic cholinergic receptor sites (Beckett, Harper & Clitherow, 1963; Belleau, 1964; Belleau & Puranen, 1963; Pauling, 1968; Robinson, Belleau & Cox, 1969).

V



VI



VII

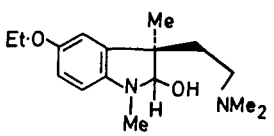
CHEMISTRY

Of the several successful syntheses of (−)-physostigmine (Julian, Pikl & Boggess, 1934; Julian & Pikl, 1935a, b; Kobayashi, 1938; Harley-Mason & Jackson, 1954), only one (Kobayashi, 1938) has been extended to use in a synthesis of (+)-physostigmine. However, in the report of that work, the salicylate of (−)-physostigmine is

quoted as been dextrorotatory, whereas it is now well established (see *e.g.* British Pharmacopoeia, 1968) that it is laevorotatory, and the salicylate of (+)-physostigmine is quoted as being laevorotatory. Furthermore, (+)-,(−)- and (±)-physostigmine were not liberated from their salicylate salts in this work and so physical data for the free bases were not obtained. Therefore Kobayashi's work leaves much to be desired.

The route chosen for the synthesis of (+)-physostigmine in the present studies is based upon the method, originally used (Julian & others, 1934; Julian & Pikl, 1935a, b) in the first synthesis of (−)-physostigmine, which appears to be more facile and affords better yields of products than the later (Kobayashi, 1938; Harley-Mason & Jackson, 1954) syntheses. (±)-5-Hydroxy-1,3-dimethyloxindole (Robinson, 1965; Longmore & Robinson, 1967) was successively *O*-ethylated, 3-cyanomethylated, catalytically hydrogenated and *N*-monomethylated, via formation of the benzylidene derivative, to afford (±)-5-ethoxy-1,3-dimethyl-3-(2-methylaminoethyl)oxindole, as already described elsewhere (Julian & others, 1934; Julian & Pikl, 1935a, b). Attempts to resolve this latter compound with (+)-camphor-10-sulphonic acid, as described earlier (Julian & Pikl, 1935b), were unsuccessful. It was therefore reduced with sodium in ethanol to afford (±)-eserethole (racemic I; R=Et, R′=Me, X=NMe), which was resolved (Kobayashi, 1938) with (+)-tartaric acid to give (+)-eserethole (VII; R=Et, X=NMe). This was de-ethylated by boiling a solution of it in light petroleum in which powdered anhydrous aluminium chloride was suspended (Julian & Pikl, 1935a, b) [the yield from this reaction was greatly increased when the reaction mixture was stirred continuously throughout the boiling period (*cf.* Hill & Newkome, 1969)] and the resulting phenol, (+)-eseroline (VII; R=H, X=NMe), was converted into (+)-physostigmine (VII; R=Me·NH·CO, X=NMe) by reaction with methyl isocyanate in the presence of a "speck" of sodium (Robinson, 1968, and refs. therein).

(−)-Physovenine (I; R=Me·NH·CO, R′=Me, X=O) has recently (Longmore & Robinson, 1966) been synthesized from (−)-eserethole (I; R=Et, R′=Me, X=NMe). By an analogous route, the (+)-eserethole (VII; R=Et, X=NMe)



VIII

prepared above was converted into its methiodide (VII; R=Et, X=$\overset{\oplus}{N}Me_2I^-$), which upon treatment with aqueous sodium hydroxide gave (−)-eserethole methine(VIII). The methiodide of this, upon treatment with boiling aqueous sodium hydroxide, yielded (+)-5-ethoxy-2,3,3a,8a-tetrahydro-3a,8-dimethylfuro[2,3-*b*]indole (VII; R=Et, X=O), which was de-ethylated by the action of anhydrous aluminium chloride to the phenol, conversion of this into (+)-physovenine (VII; R=Me·NH·CO, X=O) being effected by treatment with methyl isocyanate in the presence of a "speck" of sodium.

EXPERIMENTAL

Melting-points were measured with a Kofler hot-stage apparatus and are uncorrected. Ultraviolet spectra were measured in ethanolic solution with a Perkin-Elmer model 137 spectrophotometer, mass spectra were recorded with an A.E.I. MS. 9

spectrometer and optical rotatory dispersion spectra were obtained in 95% ethanol with a Bendix-N.P.L. "Polarmatic" spectropolarimeter. Optical rotations were measured with a Bellingham and Stanley polarimeter. Where mentioned, solutions were dried with anhydrous magnesium sulphate and solvents were removed on a steam-bath under reduced pressure (water pump). Solid analytical samples were dried (6 h) at room temperature /0·1 mm over phosphorus pentoxide.

(±)-5-*Ethoxy*-1,3-*dimethyl*-3-(2-*methylaminoethyl*)*oxindole.* This was prepared from (±)-5-hydroxy-1,3-dimethyloxindole (Longmore & Robinson, 1967; Robinson, 1965) by the reaction sequence already described (Julian & others, 1934; Julian & Pikl, 1935a).

(±)-*Eserethole.* This was prepared by reduction of (±)-5-ethoxy-1,3-dimethyl-3-(2-methylaminoethyl)oxindole with sodium in ethanol by the method previously described (Julian & Pikl, 1935a). However, the product was purified and obtained in 74% yield by column chromatography on alumina (Grade H) with ether as eluant.

Resolution of (±)-*eserethole.* This was effected by the method of Kobayashi (1938) with (+)-tartaric acid. After initial crystallization of the salt [from (±)-eserethole (20g) and (+)-tartaric acid (12g)] from dehydrated ethanol containing ether (7·5%), eight recrystallizations from ethanol containing (+)-tartaric acid (1·5%) gave fine white needles (5·77g; 36%), which exhibited constant rotation, $[\alpha]_D^{20} = +115° \pm 0·6°$ (water) and had m.p. 172–174° [lit. $[\alpha]_D^{16} = 115·5° \pm 0·7°$ (water); m.p. 173–174° (Kobayashi, 1938)].

The free base was liberated from the above salt by the addition of sodium hydroxide and was subsequently extracted into ether. After drying and removal of the solvent from the combined ethereal extracts, (+)-*eserethole* was obtained as a pale-yellow oil, which completely crystallized at 0° but which melted on warming to room temperature (20°), $[\alpha]_D^{20} = +101·5° \pm 0·7°$ (dehydrated ethanol) [lit. for (−)-eserethole, $[\alpha]_D = -81°$ (ethanol) (Polonovski, 1915), $[\alpha]_D^{28} = -81·6° \pm 0·5°$ (Julian & Pikl, 1935b)].

(+)-*Physostigmine.* This was prepared by the following modification of the method used to convert (−)-eserethole into (−)-physostigmine (Julian & Pikl, 1935b; Polonovski & Nitzberg, 1916). (+)-Eserethole (208 mg) was dissolved in sodium-dried light petroleum (b.p. 60–80°) (10ml), finely powdered anhydrous aluminium chloride (250 mg) added and the mixture boiled under reflux with continuous stirring for 10 h. After cooling, the solvent was evaporated and the residue decomposed by the addition of ice. Addition of sodium bicarbonate caused the formation of a thick gel, which, after dilution with a little water, was extracted with peroxide-free ether (4 × 20 ml). The combined ethereal extracts were washed with water (3 × 10 ml), dried and evaporated to give (+)-*eseroline* as a pale-brown oil, which partially crystallized (151 mg; 81%).

This product was immediately dissolved in sodium-dried ether (10 ml), and a "speck" of sodium added, followed by methyl isocyanate (2 ml). The reaction mixture was kept under nitrogen at room temperature (20°) with occasional shaking for three days, after which it was filtered, the filtrate evaporated and the residue subjected to column chromatography on aluminia (Grade H) with ether as eluant. A small quantity of (+)-*eserethole* was eluted with the solvent front, followed by (+)-*physostigmine* (48·0 mg; 26%), which, after evaporating the ether, was obtained as a clear glassy solid which readily crystallized as pale-yellow prisms upon trituration with a mixture of ether-light petroleum (b.p. 30–40°). Recrystallization from the solvent mixture gave white prisms (35 mg), m.p. 104–106° [lit. m.p. for the enantiomer,

106° (Polonovski & Nitzberg, 1916)], m.p. on admixture with (−)-physostigmine, 72–104°. The product had ultra-violet and mass spectra and Rf value (0·83 on a thin layer of alumina, ethyl acetate being used as solvent and iodine vapour as developer) identical with those of (−)-physostigmine, but its optical rotatory dispersion spectrum was the mirror image of that reported (Longmore & Robinson, 1969b) for (−)-physostigmine. Found: C, 65·2; H, 7·5; N, 15·1. $C_{15}H_{21}N_3O_2$ requires C, 65·5; H, 7·6; N, 15·2%.

(−)-*Eserethole methine.* This was prepared by a method analogous to that used to convert (−)-eserethole into (+)-eserethole methine (Polonovski & Polonovski, 1918, 1923a; Hoshino & Kobayashi, 1934; Longmore & Robinson, 1966). Methyl iodide (3·0 g) was added to a solution of (+)-eserethole (615 mg) in ether (6 ml). The solution immediately became turbid, and the (+)-*methiodide* gradually formed as a deposit of white plates. Recrystallization of these from acetone–ether gave white plates (940 mg; 97%), m.p. 172–174° [lit. m.p. for the enantiomer, 168–169° (Hoshino & Kobayashi, 1934); 171° (Polonovski & Polonovski, 1918); 170–171° (Polonovski & Polonovski, 1923a)]. Found: C, 49·2; H, 6·3; N, 6·9. $C_{16}H_{25}IN_2O$ requires C, 49·5; H, 6·5; N, 7·2%.

To a solution of (+)-eserethole methiodide (900 mg) in water (25 ml) was added 3N sodium hydroxide (3 ml). The solution, which immediately became turbid, was then heated (steam-bath) for ½ h. The reaction mixture, after cooling, was extracted into ether (3 × 15 ml) and the ether evaporated from the combined ethereal extracts, after drying, to afford a pale-yellow oil, which crystallized almost immediately to afford (−)-*eserethole methine* as large pale-yellow fern-like crystals (643 mg, quantitative), m.p. 83–86° [lit. m.p. for the enantiomer, 81–82° (Hoshino & Kobayashi, 1934), 80° (Polonovski & Polonovski, 1918), 89° (Polonovski & Polonovski, 1923a)], $[\alpha]_D^{20°} = -7·6°$ (dehydrated ethanol) [lit. for enantiometer, $[\alpha]_D = +10°$ (alcohol) (Polonovski & Polonovski, 1918)].

(+)-5-*Ethoxy*-2,3,3*a*,8*a*-*tetrahydro*-3*a*,8-*dimethylfuro*[2,3-b]*indole.* This was prepared by a method similar to that used in the synthesis of the enantiomer (Polonovski & Polonovski, 1918, 1923a, b; Longmore & Robinson, 1966). Methyl iodide (3 ml) was added to a solution of (−)-eserethole methine (600 mg) in ether (8 ml). After 16 h the methiodide had separated from the mixture as a pale-yellow oil, which upon further standing and trituration partially crystallized. After collection, the product, a very hygroscopic solid (900 mg; quantitative), was dissolved in water (30 ml), 7N sodium hydroxide (3 ml) added, and the solution boiled under reflux for 4 h, during which time trimethylamine was evolved and an oil separated out from the aqueous phase. After cooling, the aqueous reaction mixture and condenser washings (the product is steam-volatile) were extracted into ether (4 × 25 ml), the combined ethereal extracts washed with water (2 × 5 ml), dried and evaporated to afford a pale-yellow oil (296 mg; 60%), $[\alpha]_D^{20} = +100 \pm 1°$ (95% ethanol) [lit. for enantiometer, $[\alpha]_D^{25} = -98°$ (95% ethanol) (Polonovski & Polonovski, 1923a, b)].

(+)-*Physovenine.* Finely powdered anhydrous aluminium chloride (100 mg) was added to a solution of (+)-5-ethoxy-2,3,3a,8a-tetrahydro-3a,8-dimethylfuro[2,3-*b*] indole (77 mg) in sodium-dried light petroleum (b.p. 60–80°) (10 ml) and the mixture boiled under reflux with continuous stirring for 10 h. After cooling, the reaction mixture was "worked-up" and the product reacted with methyl isocyanate in the presence of a trace of sodium as already described in the synthesis of (±)-physovenine (Longmore & Robinson, 1967). (+)-*Physovenine* was obtained as pale-yellow prisms

(20 mg; 23%) which, after two recrystallizations from ether-light petroleum (b.p. 30°–40°), gave white prisms (12 mg), m.p. 120–122° [lit. m.p. for the enantiomer, 120–121·5° (Longmore & Robinson, 1966), 123° (Salway, 1911), 124–125° (Robinson, 1964)], m.p., on admixture with an equal weight of (−)-physovenine, 135–141° [*cf.* lit. for the racemate, m.p. 142–143° (Longmore & Roinson, 1967)]. The product had ultraviolet and mass spectra and Rf value (0·84 on a thin layer of alumina with ethyl acetate as solvent and iodine vapour as developer) identical with those of (−)-physovenine, but its optical rotatory dispersion spectrum was the mirror image of that reported (Longmore & Robinson, 1969b) for (−)-physovenine.

ENZYME STUDIES

In vitro anti-acetylcholinesterase activities were determined using erythrocyte acetylcholinesterase and the pH-stat method for measuring acetylcholine hydrolysis rates as already described (Robinson & Robinson, 1968). The results obtained are given in Table 1, in which the enzyme-inhibitor dissociation constants (K_i) of the three synthetic compounds investigated are compared with those of (−)-physostigmine and (−)-physovenine. All measurements were made after pre-incubation of the

Table 1. *Enzyme-inhibitor dissociation constants of* (−)-*physostigmine*, (−)-*physovenine, their enantiomers and* 1,1,3,3-*tetramethyl*-5-*methylcarbamoyloxyindolinium iodide.*

Inhibitor	Molor concn ($\times 10^7$)	Dissociation constants ($\times 10^7$) after pre-incubation of the inhibitor with acetylcholinesterase for 1 min
(−)-Physostigmine	1·416	5·1
(+)-Physostigmine	14·87	97
(−)-Physovenine	1·527	4·6
(+)-Physovenine	15·33	95
1,1,3,3-Tetramethyl-5-methylcarbamoyloxyindolinium iodide	0·0106	0·052

inhibitor with the enzyme for 1 min and in every case purely competitive inhibition was observed; after pre-incubation for 3 min all compounds showed mixed inhibition and after pre-incubation for 10 min the inhibition kinetics were purely non-competitive. This change in kinetics has already (Robinson & Robinson, 1968) been explained by the formation of a carbamoylated enzyme by transfer of the carbamoyl group from the inhibitor to the enzyme.

DISCUSSION

From Table 1 it can be seen that the value of K_i obtained for (−)-physotigmine and (−)-physovenine agree closely with those obtained earlier (Robinson & Robinson, 1968). Also, since a decrease in the value of K_i can be taken as an increase in inhibitory strength, (+)-physostigmine and (+)-physovenine are far less active anti-acetylcholinesterases than their natural enantiomers, whereas 1,1,3,3-tetramethyl-5-methylcarbamoyloxyindolium iodide is much more active than these alkaloids as an anti-acetylcholinesterase.

When the above alkaloids and their enantiomers are acting as anti-acetylcholinesterases, irrespective of whether the active form has ring C intact or open (see earlier), the marked differences in inhibitory activities between the members of the two enantiomeric pairs of bases is obviously caused by the optical asymmetry of the enzyme surface or receptor site or both. It could be that the difference in activity between the (+)- and (-)-isomers reflect the preferential enzymic opening of ring C in the (−)-isomers, which affords the 3*H*-indolium cations (III), the biologically active species, at or near the acetylcholinesterase receptor site.

The high anti-acetylcholinesterase activity of 1,1,3,3-tetramethyl-5-methylcarbamoyloxyindolinium iodide (IV) compared with that of (−)-physostigmine and of (−)-physovenine can be ascribed to the additional steric and/or ionic factors present in the alkaloids, or their ring C-opened 3*H*-indolium cations, at the $C_{(3)}$ atom of the indoline or 3*H*-indole nucleus, respectively, which adversely affect their binding to the receptor site. The asymmetric modification of these factors in (+)-physostigmine and (+)-physovenine, caused by optical inversion at $C_{(3)}$, further decreases the anti-acetylcholinesterase activities, probably by further adversely affecting binding of the inhibitor at the receptor.

Further studies investigating the relation between the asymmetry at $C_{(3)}$ and anti-acetylcholinesterase activity of other methylcarbamoyloxylated indoline derivatives are in progress.

Acknowledgement

We are grateful to the Medical Research Council for financially supporting this work.

REFERENCES

AHMED, M. & ROBINSON, B. (1965). *J. Pharm. Pharmac.*, **17**, 728–733.
BECKETT, A. H., HARPER, N. J. & CLITHEROW, J. W. (1963). *Ibid.*, **15**, 362–371.
BELLEAU, B. (1964). *J. mednl Chem.*, **7**, 776–784.
BELLEAU, B. & PURANEN, J. (1963). *Ibid.*, **6**, 325–328.
BERGMANN, F., WILSON, I. B. & NACHMANSOHN, D. (1950). *J. biol. Chem.*, **186**, 693–703.
British Pharmacopoeia (1968). London: The Pharmaceutical Press.
EASSON, L. H. & STEDMAN, E. (1933). *Biochem. J.*, **27**, 1257–1266.
GREIG, M. E. & HOWELL, R. S. (1948). *Proc. Soc. exp. Biol. Med.*, **68**, 352–354.
HARLEY-MASON, J. & JACKSON, A. H. (1954). *J. chem. Soc.*, 3651–3654.
HILL, R. K. & NEWKOME, G. R. (1969). *Tetrahedron*, **25**, 1249–1260.
HOOTELÉ, C. (1969). *Tetrahedron Lett.*, 2713–2716.
HOSHINO, T. & KOBAYASHI, T. (1934). *Justus Liebigs Annln. Chem.*, **516**, 81–94.
JULIAN, P. L. & PIKL, J. (1935a). *J. Am. chem. Soc.*, **57**, 539–544, 563–566.
JULIAN, P. L. & PIKL, J. (1935b). *Ibid.*, **57**, 755–757.
JULIAN, P. L., PIKL, J. & BOGGESS, D. (1934). *Ibid.*, **56**, 1797–1801.
KOBAYASHI, T. (1938). *Justus Leibigs Annln. Chem.*, **536**, 143–163.
LONG, J. P. (1963). In *Handbuch der Experimentellen Pharmakologie*, Editors: Eichler, O. & Farah, A., Ch. 8, pp. 374–427. Berlin, Göttingen & Heidelberg; Springer.
LONG, J. P. & EVANS, C. J. (1967). In *Drugs Affecting the Peripheral Nervous System*, Vol. 1, Editor: Burger, A. Ch. 6, pp. 365–379. London: Arnold; New York: Dekker.
LONGMORE, R. B. & ROBINSON, B. (1966). *Chemy Ind.*, 1638–1639.
LONGMORE, R. B. & ROBINSON, B. (1967). *Coln. Czech. chem. Commun.*, **32**, 2184–2192.
LONGMORE, R. B. & ROBINSON, B. (1969a). *Chemy Ind.*, 622–623.
LONGMORE, R. B. & ROBINSON, B. (1969b). *J. Pharm. Pharmac.*, **21**, Suppl., 118S–125S.
NEWKOME, G. R. & BHACCA, N. S. (1969). *Chem. Commun.*, 385.
PAULING, P. (1968). In *Structural Chemistry and Molecular Biology*, Editors: Rich, A. & Davidson N., p. 555. San Francisco: Freeman.

POLONOVSKI, M. (1915). *Bull. Soc. chim. Fr.*, **17**, 235–244.

POLONOVSKI, M. & NITZBERG, C. (1916). *Ibid.*, **19**, 27–37.

POLONOVSKI, M. & POLONOVSKI, M. (1918). *Ibid.*, **23**, 335–356.

POLONOVSKI, M. & POLONOVSKI, M. (1923a). *Ibid.*, **33**, 970–977.

POLONOVSKI, M. & POLONOVSKI, M. (1923b). *Compt. Rend.*, **176**, 1480–1483.

ROBINSON, B. (1964). *J. chem. Soc.*, 1503–1506.

ROBINSON, B. (1965). *Ibid.*, 3336–3339.

ROBINSON, B. (1968). In *The Alkaloids*, Vol. 10, Editor: Manske, R. H., Ch. 5, pp. 383–400. Londor. & New York: Academic Press.

ROBINSON, B. & MOORCROFT, D. (1970). *J. chem. Soc.* (*Sect. C*), in the press.

ROBINSON, J. B., BELLEAU, B. & COX, B. (1969). *J. mednl Chem.*, **12**, 848-851.

ROBINSON, B. & ROBINSON, J. B. (1968). *J. Pharm. Pharmac.*, **20**, *Suppl.*, 213S–217S.

SALWAY, A. H. (1911). *J. chem. Soc.*, **99**, 2148–2159.

STEMPEL, A. & AESCHLIMANN, J. A. (1956). In *Medicinal Chemistry*, Editors: Blicke, F. T. & Cox, R. H., Ch. 4, pp. 238–339. London: Chapman & Hall Ltd.

WILSON, I. B. & BERGMANN, F. (1950). *J. biol. Chem.*, **185**, 479–489.