3-Acetoxyquinuclidine Methiodide. Resolution, Absolute Configuration, and Stereospecificity of Interaction with the Acetylcholine Binding Sites

J. B. ROBINSON,[1] B. BELLEAU,[2]

Department of Chemistry, University of Ottawa, Ottawa, Canada

AND B. COX

Department of Pharmacology, University of Manchester, Manchester, England

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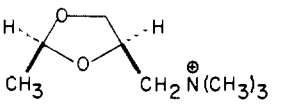
The general problem of the absolute conformation of receptor- and enzyme-bound quaternary muscarinic agents is briefly reviewed. Special attention is devoted to the super-muscarinic agent L(+)-*cis*-dioxolane (I), in which the conformation of the cationic moiety is not fixed. It is pointed out that 3-acetoxyquinuclidine methiodide has a frozen conformation about the cationic center and thus may provide a suitable conformational frame allowing a decision regarding the active conformation of the cationic part of I in the bound state. It was found that (*S*)-(−)-3-acetoxyquinuclidine methiodide (II) is related to (*S*)-(+)-β-methylacetylcholine (III) and like the latter behaves as a good substrate for AChE. As a muscarinic agent, (*S*)-(−)-II has $^1/_{600}$th the activity of acetylcholine (ACh). The (*R*)-(+) enantiomer of II was found to be inactive as a substrate for AChE, and to be $^1/_{36,000}$th as active as ACh as a muscarinic agent. It was tentatively concluded that the active conformation of I in the bound state may be as shown in IV. It seems possible, therefore, that strained conformations of stimulants may be favored in the enzyme and receptor bound states.

Evidence has accumulated in the recent past[3-6] that the configurational handedness of the muscarinic cholinergic receptor sites and the acetylcholinesterase (AChE) active centers may be similar. The most active muscarinic agent known to date[4] is (2*R*)-methyl-(4*S*)-trimethylammoniummethyl-1,3-dioxolane (I, hereafter referred to as the L(+)-*cis*-dioxolane for convenience). Its absolute configuration is the same as that of L(+)-muscarine;[7] moreover, the absolute configuration at position 4 is equivalent to that of the corresponding carbon atom in L(+)-β-methylacetylcholine.[3,8] This enantiomer is as active as ACh as a muscarinic agent[3,8] and is also the only one of the two possible isomers which acts as a substrate for AChE.[3] This suggests that both the receptor and the enzyme binding sites may share some similar configurational features. The problem of detecting specificity effects in the interaction of *non*-substrate ligands with AChE is a difficult one. A clear decision as to the possibility of a correlation between cholinergic potency and molecular events at the enzyme level must await the accessibility of suitable methods of investigation. Recently, encouraging progress in this direction has been made.[9] A comprehensive study of the thermodynamics of binding equilibria for the system AChE–quaternary salts has revealed the existence of a very large specificity effect on ΔH and ΔS binding for the L-*cis*-dioxolane (I),



I

but not for analogous dioxolanes differing in geometry or absolute configuration. It does therefore seem that the L-*cis*-dioxolane may possess the correct absolute configuration for the induction of large molecular responses in both the receptor and the enzyme. Some intriguing correlations between the physical and kinetic responses of AChE and the response of the cholinergic receptors to several classes of quaternary salts have been recently reviewed.[10] Among the numerous problems that remain to be solved, that of the absolute *conformation* of enzyme- and receptor-bound stimulants is of considerable interest. The use of constrained analogs of flexible ligands or substrates is a logical approach which is not, however, free of ambiguities owing to the persistence of residual difficulties in the elucidation of the correct conformation of the constrained molecule in solution and in the bound state. Conformations in the crystalline state can be elucidated by X-ray analysis and although intrinsically informative,[5] the results obtained by this method may not apply to molecules in solution. A clear-cut example of marked conformational disparities between the crystalline state and the in-solution state was recently given by us.[11] This work finally allowed the deduction of the absolute conformation of chymotrypsin-bound substrates after more than a decade of controversy regarding the conformation of a constrained substrate analog.[12] As it turned out, the conformation of this analog corresponds to the thermodynamically *unstable* one in the enzyme-bound state, a conclusion opposite to that which may be predicted on

(1) Postdoctoral assistant supported by the National Research Council of Canada, 1965–1966.

(2) To whom correspondence should be addressed.

(3) A. H. Beckett, N. J. Harper, and J. W. Clitherow, *J. Pharm. Pharmacol.*, **15**, 362 (1963).

(4) B. Belleau and J. Puranen, *J. Med. Chem.*, **6**, 325 (1963).

(5) P. Pauling in "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Ed., W. H. Freeman & Company, San Francisco, Calif., 1968, p 555.

(6) B. Belleau, *J. Med. Chem.*, **7**, 776 (1964).

(7) F. Jellinek, *Acta Cryst.*, **10**, 277 (1957).

(8) B. W. J. Ellenbroek and J. M. van Rossum, *Arch. Intern. Pharmacodyn.*, **125**, 216 (1960).

(9) B. Belleau and J. L. Lavoie, *Can. J. Biochem.*, **46**, 1397 (1968).

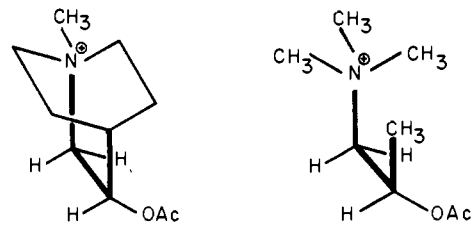
(10) B. Belleau in Proceedings of a Symposium on Drug-Receptor Interactions, Buffalo, N. Y., Aug 1968, D. J. Triggle, Ed., Academic Press, New York, N. Y., in press.

(11) B. Belleau and R. Chevalier, *J. Am. Chem. Soc.*, **90**, 6864 (1968).

(12) G. Hein, R. B. McGriff, and C. Nieman, *ibid.*, **82**, 1830 (1960).

the basis of theoretical calculations of the probable conformation of noninteracting species under vacuum.[13]

Returning to the super-muscarinic agent L(+)-*cis*-dioxolane (I), the problem of the absolute conformation of the freely oscillating cationic moiety in the bound state remains to be solved. Once this is known, the complete chirality of the binding sites may be revealed. The choice of structure for such studies is not an easy one because of the limitations outlined above. However, the results of Mashkovsky on the muscarinic activity of 3-acetoxyquinuclidine and its methiodide,[14] as well as those of Solter[15] on the ability of the latter to act as a substrate for AChE, suggested an approach to our problem because the quaternary moiety of the molecule (II) is virtually frozen in a single conformation. In addition, the bridgehead carbon atom occupies a position equivalent to that of the methyl substituent of β-methylacetylcholine (III). However, this raises the question of the configurational equivalence of the two molecules about their respective asymmetric centers. If II and III are related as shown, then only the (*S*) isomer of the former should behave as a substrate for AChE; it should also be significantly more active than the (*R*) isomer as a muscarinic agent. However, predictions of this kind are dangerous because of the distinct possibility of stereochemical inversion of specificity of the binding sites owing to special conformational effects in the ligand. Such a phenomenon has been reported by Waser[16] for D-muscarone *vs.* L-muscarine and the results have been analyzed and interpreted by us[4] in terms of an inversion of conformational stereospecificity similar to that discovered earlier for the case of chymotrypsin.[11,12] It therefore became necessary to establish the absolute configuration of the optical forms of II, their behavior toward AChE, and their potencies as muscarinic agents before attempting to use this molecule as a conformational frame for the relevant cationic part of the L-*cis*-dioxolane (I). The purpose of this communication is to report our results on this problem.



II III

Experimental Section[17]

Chemical. **(+)-3-Acetoxyquinuclidine methiodide** was prepared according to the literature[18] and recrystallized twice from EtOH; mp 164–165.5° (lit.[18] 165–166°).

Resolution of (±)-3-quinuclidinol accomplished according to the method of Sternbach and Kaiser,[19] using camphor-10-sulfonic acid. After five recrystallizations from Me_2CO–*i*-PrOH the product exhibited constant rotation, $[\alpha]^{25}D$ −1.05° (*c* 5.7, H_2O), lit.[19] $[\alpha]^{25}D$ −0.3° (*c* 3, H_2O). Decomposition of the salt gave (−)-3-quinuclidinol, $[\alpha]^{25}D$ −37.1° (*c* 3.0, 1 *N* HCl), lit.[19] $[\alpha]^{25}D$ −43.0° (*c* 3.0, 1 *N* HCl).

The mother liquors yielded crystals, $[\alpha]^{25}D$ 20° (*c* 3.3, H_2O), unchanged after recrystallizations. The free base had $[\alpha]^{25}D$ 18.5° (*c* 2.7, 1 *N* HCl); this corresponds to 75% optical purity.

(+)-3-Acetoxyquinuclidine.—(−)-3-Quinuclidinol (2 g) was acetylated with Ac_2O in C_5H_5N.[20] Fractional distillation of the product yielded 1.71 g of a fraction, bp 128–130° (28 mm), $[\alpha]^{25}D$ 28.5° (*c* 2.94, EtOH), strong band at 1725 cm^{-1} in the ir (film). *Anal.* ($C_9H_{15}NO_2$) C, H.

(−)-3-Acetoxyquinuclidine (partially resolved) was prepared as described above for the (+) isomer; $[\alpha]^{25}D$ −10.7° (*c* 2.9, EtOH). The ir spectrum was superimposable upon that of the (+) enantiomer; estimated optical purity, 70%.

(−)-3-Acetoxyquinuclidine methiodide (II) was prepared in the usual manner from (+)-3-acetoxyquinuclidine and MeI. After recrystallization from EtOH it had mp 203–204°, $[\alpha]^{25}D$ −11° (*c* 2.03, H_2O). *Anal.* ($C_{10}H_{18}INO_2$) C, H.

(+)-3-Acetoxyquinuclidine methiodide was prepared from (−)-3-acetoxyquinuclidine of 70% optical purity and MeI. After 12 recrystallizations from dry EtOH, crystals of mp 202–203°, exhibiting constant rotation were obtained; $[\alpha]^{25}D$ 11.0 (*c* 1.9, H_2O). *Anal.* ($C_{10}H_{18}INO_2$) C, H.

Absolute Configuration of (+)-3-Quinuclidinol. (a) **3-Quinuclidinyl *p*-Toluenesulfinate.**—*p*-Toluenesulfinyl chloride[21,22] (3.5 g) was dissolved in dry Et_2O (15 ml), the solution cooled to −78°, and while stirring, a solution of 2.2 g of (+)-3-quinuclidinol (75% optical purity) in a mixture of 10 ml of Et_2O and 10 ml of C_5H_5N was added over 30 min under dry N_2. After 90 min at −78°, the mixture was allowed to attain room temperature, diluted with 15 ml of Et_2O, and washed with 20 ml of 5% Na_2CO_3 in H_2O, then with H_2O. The solution was dried and evaporated to give a yellow oil (1.4 g), $[\alpha]^{25}D$ 2.0° (*c* 3.7, Me_2CO), ν_{max} (film) 1130 cm^{-1} (>S=O). It was used as such in the next step.

(b) **(−)-Methyl *p*-Tolyl Sulfoxide.**—MeMgI was prepared in Et_2O (25 ml) from MeI (1 g) and Mg (0.4 g). To this was added slowly over 20 min under N_2, a solution of the preceding quinuclidinyl *p*-toluenesulfinate (1.2 g) in 25 ml of Et_2O. After 1 additional hr, H_2O was added (10 ml) and the Et_2O solution was washed with 10% HCl, followed by 10% Na_2CO_3 and H_2O. The Et_2O was dried and evaporated to yield a yellowish oil whose ir spectrum (film) was identical with that of an authentic specimen of methyl *p*-tolyl sulfoxide. It was distilled *in vacuo*, bp 70–75° (0.15 mm) (bath temperature), to yield colorless material whose nmr spectrum ($CDCl_3$) was in agreement with the expected structure; $[\alpha]^{25}D$ −1.50° (*c* 4.49, EtOH). It follows that (+)-3-quinuclidinol possesses the (*R*) configuration.[23,24]

Enzymology.—Bovine erythrocyte AChE (Sigma) was used. The velocity of catalyzed hydrolyses was measured by the pH Stat method as previously described in detail.[25] Incubations were carried out in a total volume of 25 ml of enzyme solution previously made 0.04 *M* in $MgCl_2$ and 0.05 *M* in NaCl. The pH was maintained constant at 7.4. A CO_2-free N_2 atmosphere was maintained throughout. The system was allowed to equilibrate at 25° for 5 min prior to substrate additions. The slope of each initial velocity was taken during the second and third minute of incubation. The K_m and V_{max} values were computed from conventional reciprocal plots (Figure 1).

Pharmacology.—Terminal ileum from guinea pigs (300–400 g) was suspended in a 10-ml organ bath of Krebs solution at 36°. The usual mixture of 95% O_2 and 5% CO_2 was bubbled through the bath fluid. Contractions of the ileum were recorded on a kymograph, using an isotonic frontal writing lever (magnification 8:1 at a load of 1 g). Log concentration *vs.* response to ACh and to the 3-acetoxyquinuclidine stereoisomers were obtained using

(13) L. B. Kier, *Mol. Pharmacol.*, **3**, 487 (1967).

(14) M. D. Mashkovsky in Proceedings of the 1st International Pharmacology Meeting, Stockholm, 1962, Vol. 7, K. J. Brunings and P. Lindgren, Ed., Pergamon Press, London, 1963, p 359.

(15) A. W. Solter, *J. Pharm. Sci.*, **54**, 1755 (1965).

(16) P. G. Waser, *Pharmacol. Rev.*, **13**, 465 (1961).

(17) Melting points and boiling points are uncorrected. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

(18) C. A. Grob, A. Kaiser, and R. Renk, *Helv. Chim. Acta*, **40**, 2170 (1957).

(19) L. H. Sternbach and S. Kaiser, *J. Am. Chem. Soc.*, **74**, 2215 (1952).

(20) J. B. Kay, J. B. Robinson, and J. Thomas, *J. Chem. Soc.*, 5112 (1965).

(21) F. C. Whitmore and F. H. Hamilton, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p 492.

(22) F. Kurzer, *Org. Syn.*, **34**, 93 (1954).

(23) K. Mislow, M. M. Green, P. Laur, J. T. Melillo, T. Simmons, and A. L. Ferney, *J. Am. Chem. Soc.*, **87**, 1958 (1965).

(24) M. M. Green, M. Axelrod, and K. Mislow, *ibid.*, **88**, 861 (1966).

(25) B. Belleau and G. Lacasse, *J. Med. Chem.*, **7**, 768 (1964).

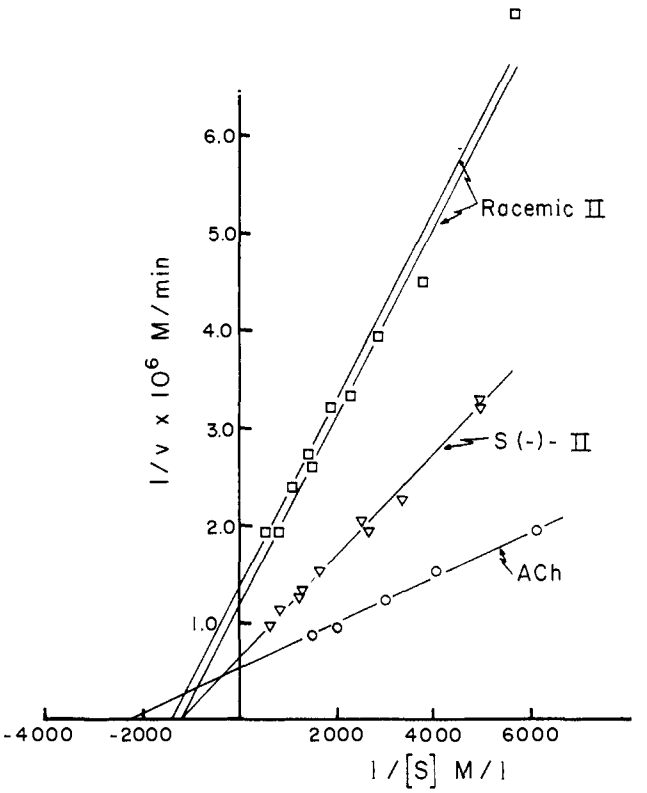


Figure 1.—Reciprocal plots for the hydrolysis of racemic and (*S*)-(−)-3-acetoxyquinuclidine methiodide (II) as compared to acetylcholine by acetylcholinesterase at pH 7.4, 25°, 0.04 *M* $MgCl_2$ and 0.05 *M* NaCl.

a 3-min dose cycle. The final curves (Figure 2) represent the calculated mean from six individual curves. The concentrations refer to the final bath concentration. Contractions produced by all agonists were inhibited by atropine at a final concentration of 10^{-9} *M*.

Results

Chemical.—The application of the asymmetric sulfoxide synthesis leads to the conclusion that (+)-3-quinuclidinol possesses the (*R*) configuration. It follows that (−)-3-acetoxyquinuclidine methiodide has the (*S*) configuration (II) which is the same as in L(+)-β-methylacetylcholine (III),[5] the stereoisomer acting as a substrate for AChE and as a muscarinic matching ACh in potency.

Enzymological.—As can be seen in Table I, (*S*)-(−)-3-acetoxyquinuclidine is a good substrate for AChE, whereas the (*R*) isomer is totally inactive in this regard. Therefore, the substrate properties of the racemic material reside exclusively in the (*S*) enantiomer. The stereospecificity of the enzyme toward this pair of enantiomers is the same as toward the β-methylacetylcholine isomers.

TABLE I
KINETIC CONSTANTS FOR THE AChE-CATALYZED HYDROLYSIS OF 3-ACETOXYQUINUCLIDINE METHIODIDE ISOMERS

Substrate	$K_m \times 10^{-4}$	$V_{max} \times 10^6$, *M*/min
ACh[a]	4.24	1.82
Racemic II[a]	8.12	0.035
(*S*)-(−)-II	7.95	1.50
(*R*)-(+)-II		Inactive as substrate

[a] The values reported by Solter[6] are at variance with these results; this author found K_m = 2.08, V_{max} = 19.27 for ACh; K_m = 1.53 and V_{max} = 6.13 for racemic II. The origin of these discrepancies may lie in that different units were used by Solter.

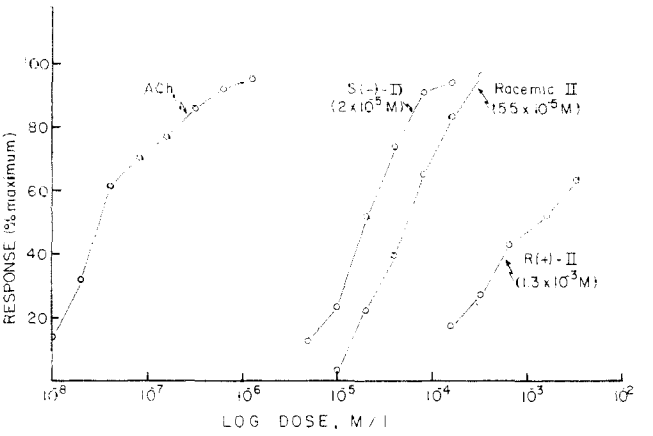
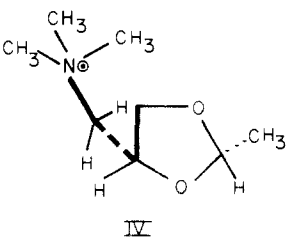


Figure 2.—Log dose-response curves for guinea pig ileum contractions induced by ACh, racemic II, (*S*)-(−)-II, and (*R*)-(+)-II; maximum response could not be induced by the latter. (*S*)-(−)-II has 1/36,000th the activity of ACh.

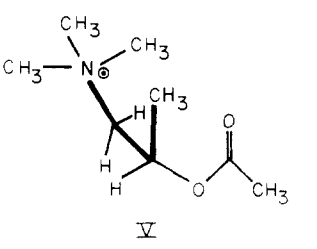
Pharmacological.—Compared to ACh (ED_{50} = 3.2 × 10^{-8} *M*), (*S*)-(−)-3-acetoxyquinuclidine methiodide (ED_{50} = 2 × 10^{-3} *M*) is 600 times weaker as a muscarinic agent. However, the (*R*) enantiomer is about 60 times weaker than the (*S*) isomer (or 1/36,000th as active as ACh). Whereas the (*S*) isomer was capable of producing maximum contractions of the ileum, the (*R*) *isomer was not*. Therefore, the activity of the racemic material resides almost entirely in the (*S*) enantiomer.

Discussion

The results described above establish that (*S*)-(−)-3-acetoxyquinuclidine methiodide (II) and (*S*)-(+)-β-methylacetylcholine (III) are stereochemically related as shown. In other words, transforming III to the conformationally constrained analog II does not lead to an inversion of stereospecificity of the type previously noted in the case of D-muscarone *vs.* L-muscarine.[4] Once more, the configurational specificities of AChE and the muscarinic receptor sites are shown to be equivalent, since both interact specifically with the (*S*) enantiomer of (−)-3-acetoxyquinuclidine methiodide. In view of these results it appears worthwhile attempting the use of II as a conformational model incorporating a constrained form of the super-muscarinic agent I. If such a comparison is made, the conclusion emerges that the cationic side chain of I should assume a conformation approximating that shown in IV in the bound state



IV



V

In addition, the active conformation of (*S*)-(+)-β-methylacetylcholine should be as in III where it can be seen that the onium functiontrimethylamm and the

β-methyl group would be nearly eclipsed in the bound state. This conclusion appears improbable on the basis of energy considerations. A skewed conformation such as in V is more appealing; a slightly twisted conformation for II is a reasonable approximation of V. If the comparisons are valid, the conclusion emerges that binding on active sites may not necessarily involve the thermodynamically preferred conformation of the ligand, a fact which was recently brought to light in the case of a constrained substrate of chymotrypsin.[11] Results of X-ray studies on crystals,[5,7] as well as theoretical calculations,[13] predict opposite conclusions; the obvious reason for this is that no account is taken of the fact that proteins display conformational specificity.[11] Owing to internal compensation effects,[9] strained conformations of substrates and inhibitors may be readily stabilized through the translocation of conformational energy within the protein. The reduction of the free energy of activation encountered in enzyme-catalyzed reactions has, in fact, been explained by Jencks[26] as resulting from the induction of a strained conformation approaching in structure that of the transition state. Isotope-effect studies on the binding of substrates on enzymes have led us to similar conclusions.[27,28] It does not seem impossible therefore that IV and V may represent the biologically active conformations at the binding site level. Finally, it is of interest to note that the configurational handedness of the AChE binding sites is similar to that of the muscarinic receptor binding sites.

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(26) W. P. Jencks, in "Current Aspects of Biochemical Energetics," N. Kaplan and E. Kennedy, Ed., Academic Press, New York, N. Y., 1966, p 273.
(27) B. Belleau and J. Moran, *Ann. N. Y. Acad. Sci.*, **107**, 822 (1963).
(28) B. Belleau, *Stud. Biophys.*, **4**, 95 (1967).

2-(N,N-Dialkylamino)ethyl Esters of α-(3-Pyridyl)mandelic Acids. Synthesis and Pharmacological Evaluation

A. NOVELLI, J. R. BARRIO,

Departamento de Quimica Orgánica

AND H. HUIDOBRO

Departamento de Farmacologia, Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires, Buenos Aires, Argentina

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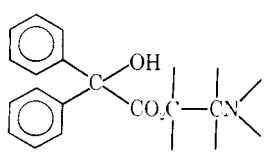
2-(N,N-Dialkylamino)ethyl esters of α-(3-pyridyl)mandelic acids were prepared and screened for pharmacological activity. Compounds VIIb and e compared favorably with benactyzine hydrochloride as inhibitors of spontaneous motility. Some of them (VIIa, b, d, and e) also show anticholinergic, spasmolytic, antihistaminic, and anti-5-HT effects.

Aminoalkyl benzilate esters (I)[1] possess pharmacological effects that have several clinical applications.[2] The presence of a pyridyl instead of a phenyl radical should change their pharmacological properties. To prove this assumption, synthesis of type II derivatives containing a 3-pyridyl radical was undertaken.



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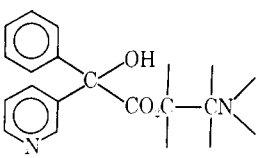
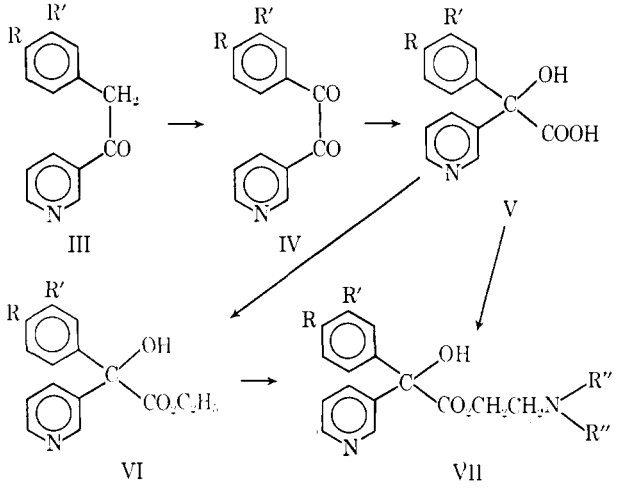


II

The general process of synthesis is shown in Scheme I. IIIa (Table I) was obtained in good yields by condensing ethyl phenylacetate and ethyl nicotinate in NaOEt.

(1) (a) A. Aström, *Acta Pharmacol. Toxicol.*, **8**, 363 (1952); (b) E. Jacobsen, *Danish Med. Bull.*, **2**, 159 (1955); (c) E. Jacobsen and Y. Shaarup, *Acta Pharmacol. Toxicol.*, **11**, 117 (1955); (d) E. Jacobsen and E. Sonne, *ibid.*, **11**, 135 (1955); (e) *ibid.*, **12**, 310 (1956); (f) H. Grethe and E. Jacobsen, *ibid.*, **13**, 125 (1957); (g) H. Holten and E. Sonne, *ibid.*, **11**, 148 (1955); (h) M. J. Raymond and C. J. Lucas, *Brit. Med. J.*, **1**, 952 (1956); (i) U. Larsen and C. H. Holten, *Acta Pharmacol. Toxicol.*, **12**, 346 (1956); (j) L. Alexander, *J. Am. Med. Assoc.*, **162**, 966 (1956).
(2) (a) I. Munkvad, *Acta Psychiat. Neurol. Scand.*, **30**, 729 (1955); (b) B. E. Davies, *Brit. Med. J.*, **1**, 480 (1956); (c) C. H. Holten, *Acta Pharmacol. Toxicol.*, **13**, 113 (1957); (d) M. A. Gardes and M. Laulan, *Presse Med.*, **65**, 180 (1957); (e) A. Coady and E. C. O. Jewesbury, *Brit. Med. J.*, **1**, 485 (1956); (f) L. Alexander, *J. Am. Med. Assoc.*, **166**, 1019 (1958).

SCHEME I



Using the same method, mono- (IIIb) and dimethoxy (IIIc) derivatives were obtained from the ethyl esters of homoanisic and homoveratric acids, respectively. Legrand and Lozac'h obtained the β-keto ester by condensing ethyl nicotinate and ethyl phenylacetate in low yields (17%) only.[3] When condensation was carried out with ethyl 3-pyridylacetate and ethyl

(3) L. Legrand and N. Lozac'h, *Bull. Soc. Chim. France*, 79 (1955).