**Table V.** Populations of the Trans Conformer (Mole Fraction, $n_t$) of Histamine and 4-Methylhistamine Monocations by Different Procedures

| Procedure | $n_t$ (histamine) | $n_t$ (4-methylhistamine) | Ref |
|---|---|---|---|
| NMR | 0.45 | 0.45 | 5 |
| EHT by internal energy difference | 0.55 | 0.75 | 3 |
| EHT by free-energy difference | 0.62 | 0.67 | 1, this work |

The only test we have is in comparing the predicted trans/gauche conformer ratios with the values found experimentally by NMR.[5] The respective populations of trans conformer monocations at 37°, predicted by EHT, were previously given[3] as 0.55 (histamine) and 0.75 (4-methylhistamine), whereas the value by NMR was the same for either, viz. 0.45 (Table V). Correspondence in the absolute values between EHT prediction and experiment must be regarded as fortuitous especially as the calculations are on isolated molecules, whereas the experiments refer to aqueous solution. What does matter is whether the two methods agree over the differences between molecules; however, as the preceding values show, there is some disagreement since the relative stability of the trans conformer was predicted to be greater for 4-methylhistamine than for histamine (by ~0.5 kcal mol$^{-1}$) but this was not reflected in the NMR results. The relative populations were predicted, however, on the assumption that the internal potential energy differences between the stable conformations could be equated to the free-energy difference. We have since shown how to refine the predictions, by allowing for the entropy content of the potential energy surface, and that for the histamine monocation this results in a modest *increase* in the predicted trans conformer population[1] (from $n_t$ = 0.55–0.62). Similar refinement for 4-methylhistamine, integrating the surface of Figure 4b around each energy minimum up to a limit of $2kT$, results in a *decrease* in the predicted trans conformer population (from $n_t$ = 0.75–0.67). Thus, taking account of the shape of the energy surface substantially reduces the extent of disagreement between the respective predictions for the two molecules (the relative stabilities now differ by only ~0.1 kcal mol$^{-1}$), in line with the experimental results. To this extent the EHT predictions are consistent. Further support for the EHT calculations comes from the agreement between calculated barriers to internal rotation derived from EHT and ab initio molecular orbital calculations.[10]

This approach appears likely to have general applicability. In principle, one may compare the conformational properties of a reference material with those of suitable congeners (which need not necessarily be methyl derivatives); differences in conformational accessibility may then be related to biological differences between the molecules. The appearance of a self-consistency within a series would permit one to define conformations "essential" to particular biological activities.

### References and Notes

(1) L. Farnell, W. G. Richards, and C. R. Ganellin, *J. Theor. Biol.*, **43**, 389 (1974) (paper 4).
(2) J. W. Black, W. A. M. Duncan, G. J. Durant, C. R. Ganellin, and M. E. Parsons, *Nature (London)*, **236**, 385 (1972).
(3) C. R. Ganellin, G. N. J. Port, and W. G. Richards, *J. Med. Chem.*, **16**, 616 (1973) (paper 2).
(4) C. R. Ganellin, *J. Med. Chem.*, **16**, 620 (1973) (paper 3).
(5) C. R. Ganellin, E. S. Pepper, G. N. J. Port, and W. G. Richards, *J. Med. Chem.*, **16**, 610 (1973) (paper 1).
(6) $N_3$ corresponds to $N^\tau$ in the recently defined nomenclature: J. W. Black and C. R. Ganellin, *Experientia*, **30**, 111 (1974).
(7) N. S. Ham, A. F. Casy, and R. R. Ison, *J. Med. Chem.*, 16, 470 (1973).
(8) B. Pullman and J. Port, *Mol. Pharmacol.*, **10**, 360 (1974).
(9) T. Yamane, T. Ashida, and M. Kakudo, *Acta Crystallogr., Sect. B*, **29**, 2884 (1973).
(10) W. G. Richards, J. Hammond, and D. H. Aschman, *J. Theor. Biol.*, in press.

# Tricyclic Quinuclidylidenes as Potential Antihistamine-Bronchodilating Agents

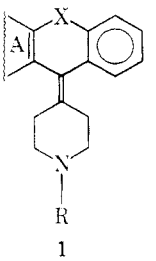
Frank J. Villani,* Thomas A. Mann, and Elizabeth A. Wefer

*Department of Medicinal Chemistry, Schering Corporation, Bloomfield, New Jersey 07003.* *Received January 23, 1975*

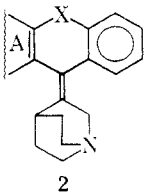
A series of quinuclidylidene derivatives of tricyclic compounds was prepared and examined for their pharmacodynamic effects. In general, the compounds showed primarily an antihistaminic effect.

Previous reports from these[1] and other laboratories[2] have described the pharmacodynamic effects of compounds containing a tricyclic moiety attached via an exocyclic double bond to an N-alkylated piperidine ring as shown in **1**. These compounds have shown potent antihistaminic, antidepressant, and anticholinergic as well as antiserotonin properties in laboratory animals and in man. It was of interest to modify the structure of **1** and replace the N-alkylated piperidine ring by a 3-quinuclidyl system as shown in **2**, especially since several naturally occurring alkaloids and other synthetic quinuclidyl derivatives have shown potent pharmacological activity.[3]

The tertiary carbinols **3** and **4** listed in Table I, required for the dehydration to **2** (Table II), were prepared by the




1

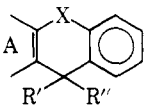



2

X = $CH_2CH_2$, CH=CH, O, S, etc.

A = phenyl, pyridyl, thienyl, etc.

**Table I**

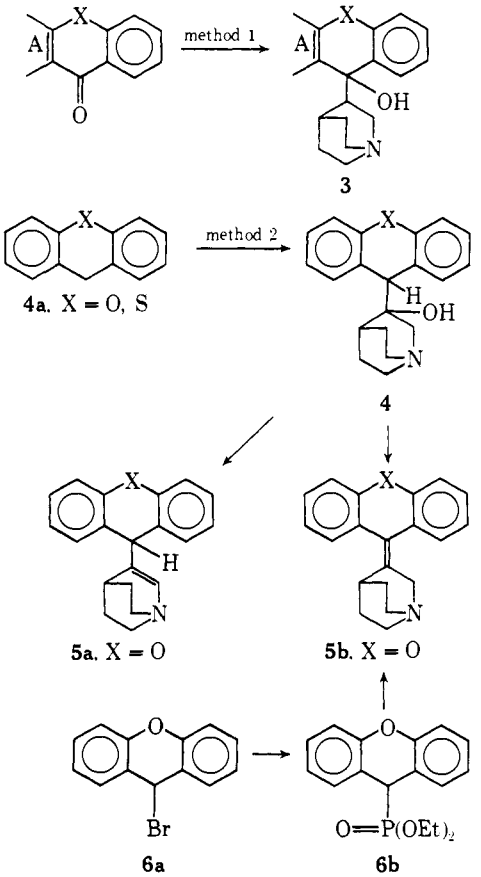


| No. | A | X | R′ | R″ | Method | Mp, °C | Rxn solvent | Yield, % | Formula[a] |
|---|---|---|---|---|---|---|---|---|---|
| 1 |  | $CH_2CH_2$ | OH | Q[b] | 1 | 157–159 | $CH_3CN$ | 52 | $C_{21}H_{24}N_2O$ |
| 2 |  | CH=CH | OH | Q | 1 | 232–233 | EtOH–$H_2O$ | 34 | $C_{22}H_{23}NO$ |
| 3 |  | O | OH | Q | 1 | 260–262 | EtOH | 49 | $C_{19}H_{20}N_2O_2 \cdot 0.5\,H_2O$[c] |
| 4 |  | O | OH | Q | 1 | 235–236 | $CH_3CN$ | 66 | $C_{19}H_{20}N_2O_2 \cdot H_2O$ |
| 5 |  | O | OH | Q | 1 | 238–241 | $CH_3CN$ | 33 | $C_{19}H_{20}N_2O_2$ |
| 6 |  | O | OH | Q | 1 | 162–165 | $CH_3CN$ | 52 | $C_{19}H_{20}N_2O_2$ |
| 7 |  | O | H | 3-OH-Q[d] | 2 | 209–210 | *i*-PrOAc | 89 | $C_{20}H_{21}NO_2$ |
| 8 |  | S | H | 3-OH-Q | 2 | 161–163 | $C_6H_6$ | 54 | $C_{20}H_{21}NOS$ |
| 9 |  | *e* | H | 3-OH-Q | 2 | 188–189 | EtOAc | 36 | $C_{20}H_{21}NO$ |

[a] All compounds were analyzed for C, H, and N. [b] Q is 3-quinuclidyl. [c] Compound isolated as hemihydrate; converted to HCl salt, mp 309–310°, from EtOH. Anal. Calcd for $C_{19}H_{20}N_2O_2 \cdot 2HCl$; C, 59.89; H, 5.81; N, 7.34. Found: C, 59.69; H, 5.83; N, 7.37. [d] 3-OH-Q is 3-hydroxy-3-quinuclidyl. [e] This is a fluorene derivative.

methods shown in Scheme I. The sodium in $NH_3$ reductive alkylation of the tricyclic ketones using 3-chloroquinuclidine (method 1) gave carbinols **3**. Reaction of the lithio derivatives of **4a** with 3-quinuclidinone (method 2) resulted in the formation of carbinols **4**. The conversion of **3** to the desired ylidene derivatives proceeded uneventfully in acidic media (*p*-TSA in $Ac_2O$ or with PPA).

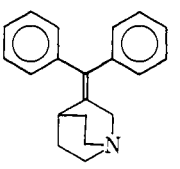
**Scheme I**




Reaction of **4** (X = O) with $SOCl_2$ followed by base resulted in the isolation of the endocyclic structure **5a**, although in poor yield. However, if the dehydration of **4** (X = O) was carried out with $SOCl_2$ in pyridine, the exocyclic structure **5b** was obtained. The structures of **5a** and **5b** were confirmed by a comparison of their NMR spectra. The xanthydryl proton ($\delta$ 4.85) and the vinyl proton ($\delta$ 6.55) in the NMR spectrum of **5a** were absent in the NMR spectrum of **5b**.

Additional support for structure **5b** was obtained by an unequivocal synthesis. Xanthydryl bromide **6a**[4] on treatment with triethyl phosphite was converted to the diethylphosphonate derivative **6b**, which on reaction with 3-quinuclidinone gave **5b** directly.

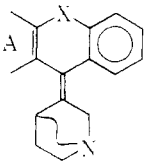
In addition to the compounds listed in Table II, the nonbridged compound **7** was prepared for biological comparison.



7

**Biological Results.** The compounds were administered orally (30 mg/kg) to five fasted guinea pigs 1 hr before exposure to an aerosol of a 0.1% solution of histamine dihydrochloride. If the test compound delayed the onset of dyspnea for 400–600 sec, it was assayed to determine the

Table II



| No. | A | X | Method | Mp, °C | Rxn solvent | Yield, % | Formula |
|---|---|---|---|---|---|---|---|
| **10** |  | $CH_2CH_2$ | 4 | 139–141 | *i*-$Pr_2O$ | 65 | $C_{21}H_{23}N_3$ |
| 11 |  | CH=CH | 3 | 324–325 | EtOH | 58 | $C_{22}H_{21}N \cdot HCl$ |
| 12 |  | O | 3 | 195–196 | *i*-$Pr_2O$ | 37 | $C_{19}H_{19}N_2O$ |
| 12 HCl | | | | 312–314 | EtOH | | $C_{19}H_{18}N_2O \cdot HCl$ |
| 13 |  | O | 4 | 232–234 | $CH_3CN$ | 66 | $C_{19}H_{18}N_2O$ |
| 14 |  | O | 4 | 218–219 | $CH_3CN$ | 63 | $C_{19}H_{18}N_2O$ |
| 15 |  | O | 4 | 140–143 | $CH_3CN$ | 57 | $C_{19}H_{18}N_2O$ |
| 16 |  | O | 5 | 196–197 | $C_6H_{14}$–hexane | 50 | $C_{20}H_{19}NO$ |
| 16 HCl | | | | 262–263 | *i*-PrOH | | $C_{20}H_{19}NO \cdot HCl$ |
| 17 |  | S | 5 | 151–152 | Hexane | 60 | $C_{20}H_{19}NS$ |
| 17 HCl | | | | 335–338 | EtOH | | $C_{20}H_{19}NS \cdot HCl$ |
| 18 |  | | 5 | 184–186 | *i*-PrOAc | 47 | $C_{20}H_{21}N$ |
| 18 HCl | | | | 316–318 | EtOH | | $C_{20}H_{21}N \cdot HCl$ |

$ED_{200}$ or that dose which would prevent the onset of dyspnea for 200 sec in 50% of 40 test animals. The most active compound in this series (Table II, No. **10**) had an $ED_{200}$ value of 620 μg/kg. Other active compounds, **11**, **15**, and **17**, had $ED_{200}$ values of 2.1, 4.03, and 3.93 mg/kg, respectively. All other compounds had only moderate to slight activity in prolonging the onset of dyspnea at the screening dose.

Compound **10** did not effect a relaxation of the isolated anaphylactic guinea pig lung indicating that this compound is acting only through an antihistaminic mechanism.

In general, the introduction of a quinuclidylidene moiety into these tricyclic systems leads to compounds having greater toxicity than their corresponding piperidylidene analogs.

## Experimental Section

All melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. NMR spectra were obtained in DMSO-$d_6$ on a Varian A-60A spectrometer using TMS as internal reference. Ir spectra were obtained on a Perkin-Elmer Model 137 spectrometer in Nujol mulls. Microanalyses were performed by the Physical Analytical Department of the Schering Corp. and the results, unless otherwise noted, were within 0.4% of the theoretical values.

**Diphenyl-3-quinuclidylcarbinol. Method 1.** Sodium metal (5.0 g, 0.2 g-atom + 10%) was added in several small portions to 300–400 ml of anhydrous liquid $NH_3$. After 10–15 min a solution of 18.2 g (0.1 mol) of benzophenone in 50 ml of anhydrous THF was added dropwise and the mixture was stirred for 20–30 min. A solution of 14.5 g of 3-chloroquinuclidine [bp 105–107° (30 mm)] in 25 ml of THF was added and stirring was continued for 2 hr. $NH_4Cl$ (20 g) was added, the $NH_3$ was allowed to evaporate, and water (200 ml) was cautiously added and then 200 ml of $Et_2O$. The crude crystalline product was filtered, washed with ether, and recrystallized from MeOH: mp 233–235° (lit.[5] mp 239°); yield 22 g (72%). Anal. ($C_{20}H_{23}NO$) C, H, N.

Alternatively, if the ketone to be alkylated was insoluble in THF, it could be added as a THF suspension or could be added to the liquid $NH_3$ without any solvent. In many cases after decomposition ($NH_4Cl$ and $H_2O$), the product was extracted ($CHCl_3$) and washed, the solvent was removed, and the residue was triturated with petroleum ether (bp 60–90°) or with hexane.

**5-(3-Quinuclidylidene)-2-methoxyxanthene.** Using method 1, this compound was obtained directly from 20.1 g (0.09 mol) of 2-methoxy-5-xanthone: yield 9.9 g (33%); mp 149–150° from $CH_3CN$; uv $\lambda_{max}$ 250 mμ (ε 12,500), 315 (10,000). Anal. ($C_{21}H_{21}NO_2$) C, H, N.

**5-(3-Hydroxy-3-quinuclidyl)xanthene. Method 2.** A solution of *n*-butyllithium was prepared at −10° from 10.5 g of lithium (1.5 g-atoms) and 102.8 g (0.75 mol) of *n*-butyl bromide in approximately 500 ml of $Et_2O$. To this reagent at −10°, a solution of 91 g (0.5 mol) of xanthene in 400 ml of dry THF was added dropwise, followed after 1 hr by a solution of 63 g (0.5 mol) of 3-quinuclidone in 200 ml of THF. The mixture was allowed to warm to room temperature and stirred overnight, decomposed with $H_2O$, extracted ($CHCl_3$), and washed and the solvent was removed. The residue was processed as shown in Table I.

**Benzhydrylidene-3-quinuclidine (7). Method 3.** A solution of 7.2 g (0.025 mol) of carbinol, 7 g of *p*-TSA, and 150 ml of $Ac_2O$ was heated on the steam bath for 5 hr and the excess solvent removed in vacuo. The residue was suspended in $H_2O$, basified ($NH_4OH$), extracted ($CHCl_3$), and washed, the solvent was removed, and the residue was recrystallized from hexane: yield 5 g (83%); mp 139–140°; uv $\lambda_{max}$ 253 mμ (ε 14,000). Anal. ($C_{20}H_{21}N$) C, H, N.

**Dehydration Method 4.** The carbinol from Table I was heated with stirring at 140–145° for 16 hr with 40 times its weight of PPA. The warm mixture was poured into ice, basified with NaOH, extracted ($CHCl_3$), and washed, the solvent was removed, and the residue was recrystallized.

**Dehydration Method 5.** To a solution of 18.4 g (0.06 mol) of carbinol **7** (Table I) in 200 ml of dry pyridine was added dropwise

with cooling (0–5°) and stirring a solution of 5 ml of thionyl chloride in 10 ml of pyridine. After 1 hr the mixture was heated under reflux for 2 hr and cooled to room temperature, 50 ml of 50% NaOH was added, and the heating was continued for an additional hour. The mixture was poured into $H_2O$, extracted ($Et_2O$), and washed, the solvent was removed, and the residue was recrystallized several times.

**3-(5-Xanthyl)-2,3-dehydroquinuclidine (5a).** A mixture of 9.3 g (0.03 mol) of carbinol **7** (Table I) and 50 ml of thionyl chloride was heated on the steam bath for 2 hr and the excess solvent removed in vacuo. The residue was dissolved in $H_2O$, 50 ml of 25% NaOH solution was added, and the mixture was heated at 80° for 1 hr. After cooling, the product was extracted ($Et_2O$) and washed and $Et_2O$ was removed. The residue was recrystallized from *i*-$Pr_2O$: yield 1.5 g (17%); mp 117–119°. Anal. ($C_{20}H_{19}NO$) C, H, N.

**Alternate Synthesis of 5b.** To 5.3 g (0.02 mol) of 5-bromoxanthene[5] was added 3.6 g of triethyl phosphite. There was an immediate reaction which was moderated by cooling. The mixture was allowed to stand overnight at room temperature and then heated under reflux for 1 hr, and the low-boiling materials were removed in vacuo on a steam bath. To the light yellow viscous residue, 6 ml of DMF and 1.5 g of NaOMe were added, followed by the dropwise addition of a solution of 2.6 g of 3-quinuclidinone in 15 ml of DMF. The mixture was stirred at room temperature for 2 hr and poured into $H_2O$ and the product was filtered and recrystallized from benzene–hexane: yield 2.4 g (49%); mp 196–197°.

**Acknowledgments.** The biological data reported herein were obtained by Mr. Salvatore Tozzi and his staff of the Department of Pharmacology of the Schering Corp. The assistance of Mr. James Morton of the Physical Analytical Services Department of the Schering Corp. in the interpretation of the NMR spectra is gratefully acknowledged.

## References and Notes

(1) (a) F. J. Villani, P. J. L. Daniels, C. A. Ellis, T. A. Mann, K. C. Wang, and E. A. Wefer, *J. Med. Chem.*, **15**, 750 (1972); (b) F. J. Villani, T. A. Mann, E. Wefer, J. Hannon, L. L. Larca, M. J. Landon, W. Spivak, D. Vashi, S. Tozzi, G. Danko, M. delPrado, and R. Lutz, *ibid.*, 18, 1 (1975).
(2) See, for example, and references therein: (a) E. L. Engelhardt, H. C. Zell, W. S. Saari, M. C. Christy, C. D. Colton, C. A. Stone, J. M. Stavorski, H. C. Wenger, and C. L. Ludden, *J. Med. Chem.*, 8, 829 (1965); (b) J. M. Bastian, A. Ebnöther, E. Jucker, E. Rissi, and A. P. Stoll, *Helv. Chem. Acta*, **49**, 214 (1966); (c) E. L. Engelhardt and H. C. Zell, U.S. Patent 3,275,640 (1966); (d) E. E. Galantay, U.S. Patent 3,458,522 (1969).
(3) L. N. Yakhontov, *Adv. Heterocycl. Chem.*, 11, 473 (1970).
(4) M. Gomberg and L. H. Cone, *Justus Liebigs Ann. Chem.*, **376**, 192 (1910).
(5) R. Tondeur and M. Urbain, *Chim. Ther.*, 207 (1966).

# Synthesis and Biological Activity of Spin-Labeled Analogs of Biotin, Hexamethonium, Decamethonium, Dichlorisoproterenol, and Propranolol

Birandra K. Sinha† and Colin F. Chignell*

*Section on Molecular Pharmacology, Pulmonary Branch, National Heart and Lung Institute, Bethesda, Maryland 20014.*
*Received November 25, 1974*

Spin-labeled analogs of biotin (vitamin H), hexamethonium, decamethonium, dichlorisoproterenol, propranolol, and primaquine containing the nitroxide free radical have been synthesized and tested for biological activity. The four spin-labeled analogs of biotin, 4-biotinamido-2,2,6,6-tetramethyl-1-piperidinyloxy (IV), 3-biotinamido-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (V), 3-biotinamidomethyl-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (VI), and 4-(biotinylglycyl)amino-2,2,6,6-tetramethyl-1-piperidinyloxy (VII), all interacted with avidin, a specific biotin binding protein found in raw egg white, at the same sites as did biotin itself. An unsymmetrical decamethonium spin label (XVIII) in which one of the quaternary methyl groups had been replaced by the 4-(2,2,6,6-tetramethyl-1-piperidinyloxy) moiety was 13 times more potent as an inhibitor of *Torpedo californica* acetylcholinesterase than the parent drug. The symmetrical decamethonium (XVI) and hexamethonium (XIV) spin labels were 18 and 1.8 times as active as decamethonium in the same assay system. The substitution of the 4-(2,2,6,6-tetramethyl-1-piperidinyloxy) group for the isopropyl groups of β-adrenergic blocking drugs dichlorisoproterenol and propranolol, to give spin labels XXI and XXII, caused a 45 and 54% reduction, respectively, in the ability of these compounds to inhibit the isoproterenol-stimulated activity of rat fat cell membranes. Finally, modification of primaquine by the introduction of the 4-(2,2,6,6-tetramethyl-1-piperidinyloxy) substituent into the amino group of the butyl side chain completely abolished the ability of the drug to bind to nucleic acids. These results suggest that the incorporation of the nitroxide group into drug molecules may be a useful approach to the synthesis of more specific spin labels for biological systems, such as egg white avidin, acetylcholinesterase, and the β-adrenergic receptor.

During recent years spin-labeled drug molecules have played an increasingly important role in studies of the interaction of drugs with their cellular targets or "receptor" macromolecules.[1-5] Spin-labeled drugs have also been used to determine the levels of morphine, barbiturates, sulfonamides, and other drugs in biological fluids.[6,7] The most commonly employed spin labels utilize the nitroxide group, since this free radical is very stable in aqueous solutions at physiological pH values. In an earlier paper,[8] we described procedures for synthesizing spin-labeled analogs of sulfonamides, acetylcholine, and barbituric acid. We now report the synthesis of spin-labeled analogs of (a) the vitamin biotin (IV–VII) for studies of egg white avidin, (b) decamethonium and hexamethonium (XIII–XIX) as potential probes for acetylcholinesterase and the acetylcholine receptor, (c) the β-adrenergic blocking drugs propranolol (XXII) and dichlorisoproterenol (XXI) for studies of the β-receptor and its interrelationship with membrane-bound adenylyl cyclase, and (d) the antimalarial drug primaquine (XXIII) for binding studies with nucleic acids.

The synthesis of the biotin analogs IV–VII was achieved by condensing the corresponding spin-labeled amines, prepared by previously reported methods, with biotin in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). For example, V was prepared by the condensation of biotin with amine II. We were unable to prepare biotinylglycine using either the biotinyl acid chloride technique described by Woli and coworkers[9] or the mixed an-

† Visiting Fellow in Pulmonary Branch, NHLI, NIH, Bethesda, Md.