thiophene (1.0 g, 2.88 mmoles) in 20 ml of dry C_6H_6 was treated with piperidine (0.29 ml, 2.88 mmoles) and allowed to stir at room temperature for 2 weeks. C_6H_6 was removed under reduced pressure, yielding a yellow solid which was dissolved in H_2O (10 ml), treated with excess 10% NaOH, and gently warmed for 1 hr. The reaction mixture was then poured into H_2O (100 ml) and the pH was adjusted to 7 with dilute HCl. The aqueous solution was extracted with four 50-ml portions of ether, and the combined ether extracts were dried (Na_2SO_4) and treated with dry HCl, producing an oil. The oil solidified readily under vacuum, and was recrystallized from EtOH-$CHCl_3$ to yield 0.48 g (58%) of thick white crystals: mp 252–253.5° dec; ν_{max}^{KBr} 3.10 (H-bonded phenolic OH), 3.7–3.9 (HN$^+$), and 6.23 μ (C=C aromatic). *Anal.* ($C_{13}H_{13}ClNOS$) C, H, N.

Acknowledgments.—We wish to acknowledge support of the U. S. Public Health Service by research Grant GM 10366 to Indiana University and fellowship GM-24,364-02 to T. R. B. We are also grateful to Dr. E. A. Zeller of Northwestern University Medical School and Dr. R. A. Beek of the New Jersey Neuro-Psychiatric Institute for providing biological screening data.

Adamantoyl Esters of Pyridoxol[1]

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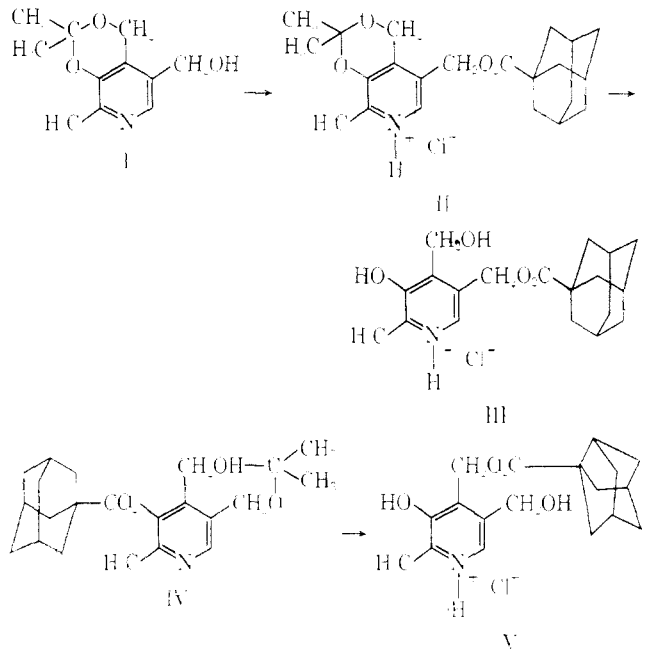
Received September 2, 1967

Gerzon and his coworkers[2-5] have shown that introduction of the adamantane group imparts interesting biological properties to representative compounds of various classes. Similarly, Zakrzewski, *et al.*,[6] found that N-adamantyl-*p*-aminobenzamide was an inhibitor of *Escherichia coli*. Hydrophobic binding of the adamantane moiety to the receptor site has been invoked as a probable explanation for the biological activity of the various adamantane derivatives.[3]

In order to investigate the chemical and biological usefulness of the adamantoyl group in vitamin B_6 chemistry and pharmacology, we prepared some adamantoates of pyridoxol (Scheme I). Of particular interest to us is their potential utility in probing for hydrophobic regions within the receptor sites at which pyridoxol analogs bind. The possibility of the existence of such regions suggested itself in the course of our previous studies.[2]

Methods for the selective introduction of the adamantoyl group into the α^5 and α^4 positions of pyridoxol are indicated in Scheme I and utilize the two isomeric isopropylidene derivatives[7,8] of pyridoxol as starting

SCHEME I



materials. Adamantoylation by adamantoyl chloride had to be carried out under more vigorous conditions than the similar reactions with other acyl chlorides,[7,8] indicating steric hindrance. Nevertheless, 3-O-adamantoyl-α^5,α^5-isopropylidenepyridoxol (IV) was found to rearrange to give the α^4-O-ester V; in this respect, adamantoyl does not appear to differ from other acyl groups, although it is conceivable that the bulk of the adamantoyl group could interfere with the formation of the orthoacid intermediate during the rearrangement.[9] The structures of the resulting esters have been confirmed by nmr, ir, and uv spectroscopy. The free phenolic hydroxyl in α^4-O-adamantoylpyridoxol is indicated by a positive Gibbs test and by characteristic shifts in the uv spectra in acidic and basic solutions.[9]

Preliminary evaluation of the α^4- and α^5-adamantoates (III and V) with *Saccharomyces carlsbergensis* (ATCC 9080) indicates that they are comparatively weak growth inhibitors, producing approximately half-maximal growth at 10^{-4} *M*.[10]

Experimental Section

Where analyses are indicated only by symbols of elements, analytical results obtained for those elements were within 0.3% of the theoretical values.

α^4,3-O-Isopropylidene-α^5-O-adamantoylpyridoxol (II).—To a stirred solution of α^4,3-O-isopropylidenepyridoxol[7] (I, 0.50 g) in 5 ml of anhydrous pyridine, adamantoyl chloride (1.0 g) in 3–4 ml of pyridine was added. Stirring was continued for 24 hr and then the mixture was refluxed for 0.5 hr. (The reaction was only partially complete after 4 hr.) Water (few drops) was introduced, the mixture was stirred for 1 hr, poured into ice water (50 ml), let stand overnight, and extracted with ether. The ether extract was washed (Na_2CO_3, H_2O) and dried ($CaSO_4$). Evaporation of the ether solution *in vacuo* left an oil, from which 0.81 g (83%) of the crude hydrochloride (mp 140–160°) was obtained by the addition of anhydrous ethereal HCl. Recrystallization from C_6H_6–ether raised the melting point to 173–174.5°. *Anal.* ($C_{22}H_{30}ClNO_4$) C, H, N.

α^5-O-Adamantoylpyridoxol Hydrochloride (III).—α^4,3-O-Isopropylidene-α^5-O-adamantoylpyridoxol (II, 0.177 g) was re-

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fluxed in 0.2 *N* methanolic HCl (15 ml, prepared by diluting 1.0 *N* HCl with MeOH). The solution was evaporated to dryness *in vacuo*, and the semicrystalline residue crystallized (EtOH–Et_2O). A yield of 0.099 g (61%) of crystalline material was obtained; mp 173–175° (a different crystalline form has mp 158–160°); λ_{max} 291 mμ (ϵ 9.1 × 10^3) in 0.1 *N* HCl; λ_{max} 244 mμ (ϵ 6.2 × 10^3), 309 mμ (ϵ 6.6 × 10^3) in 0.1 *N* NaOH. *Anal.* ($C_{19}H_{26}ClNO_4$) C, H.

α^4,α^5-O-Isopropylidine-α^3-O-adamantoylpyridoxol Hydrochloride (IV).—α^4,α^5-O-Isopropylidenepyridoxol[8] (750 mg) was dissolved in 25 ml of pyridine and 1 g of adamantoyl chloride in 25 ml of pyridine was added. After refluxing for 0.5 hr and evaporation *in vacuo*, the residue was extracted with ether, filtered, dried, and treated with ethereal HCl. The hydrochloride was filtered and was washed (Et_2O), yield 1.47 g, mp 176–180° which was raised to 183–184° after recrystallization from C_6H_6–Et_2O. *Anal.* ($C_{22}H_{30}ClNO_4$) C, N.

The free base had mp 140–142° (from petroleum ether, bp 37–54°).

α^4-O-Adamantoylpyridoxol Hydrochloride (V).—α^4,α^5-O-Isopropylidene-α^3-O-adamantoylpyridoxol (free base from IV, 548 mg) was dissolved in methanolic HCl containing 10% H_2O, and was heated at 65–70° for 1 hr. The solution was evaporated to dryness, the residue was dissolved in EtOH, and the resulting solution was evaporated again. After recrystallization (EtOH–Et_2O), the yield was 373 mg (76%); mp 182–183°; λ_{max} 293 mμ (ϵ 8.3 × 10^3) in 0.1 *N* HCl; λ_{max} 243 mμ (ϵ 6.4 × 10^3), λ_{max} 309 mμ (ϵ 6.8 × 10^3) in 0.1 *N* NaOH. *Anal.* ($C_{19}H_{26}ClNO_4$) C, H. When the hydrolysis was conducted in 0.1 *N* aqueous HCl, the yield was reduced to 17%.

Acknowledgments.—This study was supported by a research grant (CA-08793) from the National Cancer Institute, U. S. Public Health Service. We wish to thank Dr. Charles A. Nichol for his encouragement and interest.

Isoquinolines as Cholinesterase Inhibitors. I

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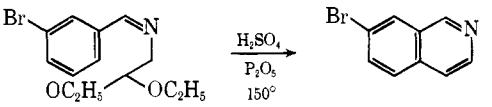
Received April 3, 1967
Revised Manuscript Received September 13, 1967

In our synthetic work on isoquinolines, it became apparent that any cholinesterase inhibitory properties of these molecules may be of interest in comparison with simple aliphatic or monocyclic amines. Although bis-quaternary quinolines[1] and isoquinolines[2] have been evaluated as cholinesterase inhibitors, tetrahydro- and decahydroisoquinolines have not been reported to be active. Several substituted isoquinolines and their hydrogenated derivatives have been prepared and evaluated against human plasma cholinesterase in an initial screening program which will precede further study.

The synthesis of isoquinolinecarboxylic acids, by way of a Pomeranz–Fritsch ring closure of 3-bromobenzalaminoacetal, as outlined by Tyson,[3] was followed. However, instead of a mixture of 5- and 7-isoquinolinecarboxylic acids being produced, as reported by Tyson, we were able to isolate only one product. In order to identify the product from the above synthesis, 5-isoquinolinecarboxylic acid was synthesized from 5-nitroisoquinoline as outlined in the Experimental Section. Mixture melting point, analytical, and gas chromatographic data of a number of derivatives of 5-carbomethoxyisoquinoline and the corresponding derivative, prepared by the Tyson synthesis, indicated that the Pomeranz–Fritsch ring closure in the Tyson synthesis had taken place specifically at the 6 position of the 3-bromobenzalaminoacetal, resulting, exclusively, in the formation of 7-isoquinolinecarboxylic acid.



The tetrahydro and decahydro derivatives of the methyl esters of both the 5- and 7-isoquinolinecarboxylic acids were prepared as described in the Experimental Section. Complete hydrogenation under low-pressure conditions proceeded smoothly;[4] more difficulty was experienced in hydrogenating the 5-methyl ester to the decahydro derivative than the corresponding 7 isomer. The 5-acetoxy derivatives were synthesized to evaluate the influence of position and nature of the carboxy group on cholinesterase inhibition. During the complete hydrogenation of 5-acetoxy-2-ethylisoquinolinium bromide (I) in glacial acetic acid containing sulfuric acid, hydrogenolysis and condensation of two molecules of the resulting decahydro alcohol occurred yielding a bis(2-ethyldecahydroisoquinoline) ether. This finding complements our evidence outlined in an earlier communication.[4] Furthermore, conversion of I to its corresponding quaternary hydroxide, followed by hydrogenation under identical conditions, yielded 5-hydroxy-2-ethyldecahydroisoquinoline, substantiating our earlier contention[4] of the involvement of the halide ion in this ether condensation.

Biological Activity.—Four series of substituted isoquinolines were screened as cholinesterase inhibitors. Manometric determinations were carried out on a GME-Lardy RWB-3 Warburg instrument at concentrations of 1 × 10^{-3} *M*. Further details of the procedure have been outlined by Beasley, *et al.*[5] For the synthesis of compounds not reported here see ref 4. The biological results are shown in Tables I and II. The data in Tables I and II indicate that the hydrogenated compounds possess greater inhibitory properties, the greatest inhibition being associated with the fully saturated compounds. This trend may involve the "semiflexible" nature of the more saturated compounds as compared with the more rigid flat structures associated with the unsaturated compounds as well as the greater degree of hydrophobicity of the more saturated molecules. Evidence for some hydrophobic sites in adenosine deaminase has recently been presented[6] and Augustinsson's[7] work may imply the presence of similar sites in cholinesterase. The significant increase in enzyme inhibition produced by the 3,4,5-trimethoxybenzoyl esters compared with the corresponding free hydroxy compounds is evident (Table I). Derivatives of the isoquinolinecarboxylic acid (Table II) do not seem to possess any significant ac-

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