GLC Determination of New Antiarrhythmic Drug

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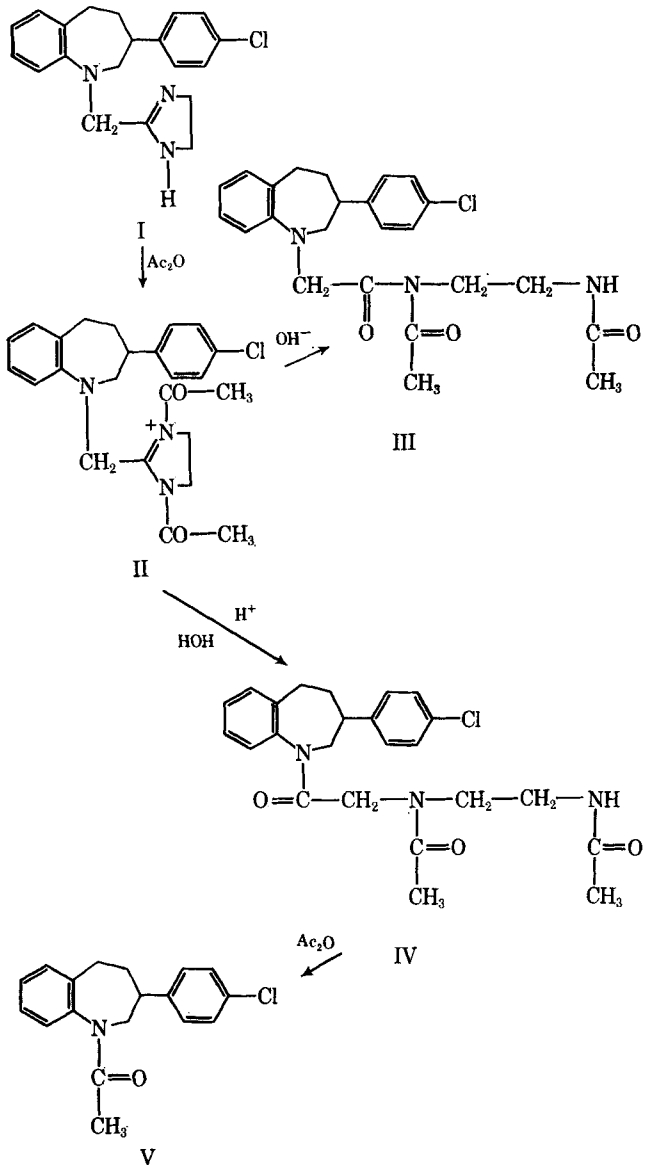
Abstract □ A GLC method for the determination of a new antiarrhythmic compound is described. In an attempt to define the compound being chromatographed, isolation and identification techniques were carried out on the derivative and a mechanism for the unusual reaction is proposed.

Keyphrases □ 3-(*p*-Chlorophenyl)-2,3,4,5-tetrahydro-1-[(2-imidazolin-2-yl)-methyl]-1-benzazepine—GLC method of microgram quantification □ Derivative formation, antiarrhythmic drug—isolation, identification, mechanism □ GLC—determination, antiarrhythmic drug

The clinical introduction of a new antiarrhythmic compound, 3-(*p*-chlorophenyl)-2,3,4,5-tetrahydro-1-[(2-imidazolin-2-yl)-methyl]-1-benzazepine (Compound I, Scheme I) has made it necessary to develop methodology capable of detecting microgram quantities of the compound for pharmacokinetic studies.

Preliminary work indicated that Compound I could not be suitably chromatographed as the parent compound. Since Tishler *et al.* (1) showed previously that similar compounds could be acetylated at elevated conditions, this approach was undertaken and found to yield, at around 140°, a derivative suitable for quantitation work at the micro level. In an attempt to define the derivative, the reaction product was isolated and characterized by physical and chemical means, thereby leading to the proposed mechanism in Scheme I.



Scheme I—Proposed mechanism for the derivatization of 3-(p-chlorophenyl)-2,3,4,5-tetrahydro-1-[(2-imidazolin-2-yl)-methyl]-1-benzazepine

EXPERIMENTAL

Apparatus—GLC determinations were carried out on an instrument (Barber Colman model 5000) equipped with glass columns [2.44 m. (8 ft.) × 4 mm. i.d.], containing 1% polyethylene glycol (Carbowax) 20 M on 100–120 mesh Gas Chrom S, and a flame-ionization detector. The operating parameters were: nitrogen flow rate, 40 ml./min.; temperature of column, 235°; temperature of detector, 250°; temperature of flash heater, 250°; and electrometer sensitivity, 3×10^{-11} amp. full-scale.

Procedure—Five milliliters of the standard solution containing 0.5 mcg. of the HCl salt and 5 ml. of the sample solution are made alkaline with 0.4 ml. of 6 *N* sodium hydroxide. The samples are extracted twice with 5-ml. portions of a mixed solvent containing 10% methylene chloride, spectroquality, in ether. The appropriately combined extracts are evaporated to dryness with the aid of a stream of nitrogen in a 10-ml. centrifuge tube. The residues are reacted with 0.1 ml. of a 1:1 mixture of acetic anhydride–pyridine in a capped centrifuge tube for 20 min. in a 135° oil bath. The samples are again evaporated to dryness under a stream of nitrogen.

The residue is diluted with 10 μl. of dimethylformamide, and a 2-μl. sample is chromatographed. The concentration of the sample solution is obtained by graphically comparing its area (height and width at height/2) to the area of the standard solution. The response of the standard to the flame-ionization detector is linear over the microgram range needed.

For identification purposes, milligram quantities of the derivative were prepared as already described. The residue obtained was shown by TLC to be essentially one spot and therefore was used for characterization purposes without further purification.

RESULTS AND DISCUSSION

Based on earlier data generated by Tishler *et al.* (1), one would normally anticipate that either Compound III or IV would be the compound being chromatographed. However, UV, IR, NMR, and mass spectra data clearly indicated that the derivative was singly acetylated and that the acetyl group was connected to the nitrogen of the seven-membered ring.

To validate completely the proposed structure, Compound V was synthetically prepared by an alternate route. The various physical parameters, including gas chromatography retention time, obtained on the synthesized compound were identical to those of the residue isolated in the acetylation reaction, thereby confirming the proposed structure, V.

Although several pathways for the formation of Compound V can be proposed, the one shown in Scheme I is favored, based on data previously generated by Tishler *et al.* (1) on related morphanthridine compounds. The amide interchange (Compound IV to Compound V) was also reported by Rodionov and Zvorykina (2)

in their work on acylation reactions (Scheme II):

$$R{-}CH_2{-}CO{-}NH{-}Ph \xrightarrow[Ac_2O]{reflux} Ac{-}NH{-}Ph$$

Scheme II

The application of the gas chromatographic procedure was applied to plasma samples. A more detailed discussion (data substantiating the selection of the various parameters described under *Procedure*) of this application for pharmacokinetic studies will be presented at a later date.

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pH Dependence of Fluorescence of Riboflavin and Related Isoalloxazine Derivatives

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Abstract □ pKa values were determined for several flavins by absorptiometry and by fluorimetry. The poor agreement between absorptiometrically and fluorimetrically determined pKa values is attributed to the fluorimetrically measured quantities being due to equilibria in the fluorescent state (lowest excited singlet state). The excited-state pKa's and the ground-state pKa's do not correspond to protonation at the same sites in the isoalloxazine ring. The significance of excited-state equilibria for fluorimetric analysis is considered.

Keyphrases □ Fluorescence, pH dependence—riboflavin, related isoalloxazine derivatives □ Riboflavin, related isoalloxazine derivatives—fluorescence pH dependence □ pH dependence—fluorescence of riboflavin, related isoalloxazine derivatives

Fluorimetric assay is probably the most widely employed method for the determination of riboflavin (1). The intense green fluorescence of riboflavin (2) and that of its photodecomposition product lumiflavin (3–5) have been used in quantitative analysis.

Riboflavin fluorescence is constant in intensity between pH 3 and 8 but is quenched at pH <3 and >8. As a result, it is necessary to maintain pH 3–8 in solutions upon which riboflavin assay is performed. Early studies of the fluorescence intensity of riboflavin as a function of pH yielded two pKa values of 2 and 10 (6). However, absorptiometric determination of the pKa's in the same acidity regions gave values of -0.2 and 9.8 (7). Subsequent measurements of the second pKa of riboflavin by electrochemical methods yielded values of 9.9 (8) and 10.0 (9). In light of the good agreement between the fluorimetrically, absorptiometrically, and electrometrically determined values of the second pKa, it seems reasonable to conclude that they all correspond to the same dissociation. However, the substantial difference between the value of the first pKa as determined by fluorimetric titration and that determined by absorptiometry suggests that the two pKa values do not correspond to identical protolytic processes.

The most obvious explanation for the latter dilemma is that the absorptiometric measurements yield a conventional pKa value corresponding to a protolytic dissociation in the ground state of the molecule, while the fluorescence measurements yield a pKa corresponding to a protolytic reaction of riboflavin in the electronically excited state from which fluorescence originates: the lowest excited singlet state (10). Hereafter, pKa values corresponding to electronically excited states will be referred to as pKa*. Prototropism in the lowest excited singlet state derives from the fact that rates of protonation and dissociation are often fast enough to compete with fluorescence for deactivation of the excited state of a fluorescent molecule. Consequently, the fluorescence intensity may reflect the relative concentrations of acid and conjugate base in the electronically excited state rather than the corresponding quantities in the ground state (as determined by absorptiometry or electrometry). Because the electronic distribution is generally different in an electronically excited molecule from that in the ground state of the same molecule, the binding energy of a proton and hence the pKa is different from that in the ground-state molecule. Obviously, it is as important to know pKa* values in fluorimetric analysis as it is to know pKa values in absorption spectrophotometric analysis in order to establish the pH range of optimum analytical utility.

To investigate more closely the differences between the pH dependencies of the fluorescence spectra and