Acetonyl Esters of Hydroxybenzoic Acids As Potential Antisickling Agents

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SUMMARY

Based on the known mechanism of action of some antisickling agents, a novel hypothesis is advanced to support our view that acetonyl esters of some hydroxybenzoic acids might serve as effective antisickling agents. By a subtle though significant modification of the Schwyzer's method (1955) for the synthesis of activated esters of carboxylic acids, three new acetonyl esters, i.e., acetonyl p-hydroxybenxoate (III), acetonyl p-acetoxybenzoate (IV), and acetonyl salicylate (V) were synthesized and fully charac-

INTRODUCTION

terized.

Since the implication of p-hydroxybenzoic acid, vanillic acid, m-hydroxybenzoic acid and o-hydroxymethyl benzoic acid as the anti-sickling principles in the root of Fagara zanthoxyloides (El Said et al., 1971; Sofowara and Isaacs, 1971; Sofowara, 1975) other derivatives of benzoic acid, such as p-aminobenzoic (Baker, 1975) and acetylsalicylic acid (Aspirin) (Fameil and McMeekin, 1973) have been reported to reveal antisickling properties, Aspirin, in particular, has been reported to be capable of acetylating haemoglobin S (HbS) both in vitro and in vivo though with no discernible effect on the sickling process (de Furia et al., 1973; Bridges et al., 1974).

Various chemical approaches toward rectifying the behaviour of HbS, based on both covalent and non-covalent modification have been tried. Significant in this line of approach is the influence of carbamyiation of HbS—brought about by the cyanates—on sickling (Gilette et al., 1971; Manning et. al., 1972).

ACETONYL ESTERS AS POTENTIAL ANTISICK-LING AGENTS.

Based on the chemistry of acetonyl esters (a group of activated esters of carboxylic acids with no previously reported biological or medicinal action), a postulate is going to be advanced to buttress our novel opinion that there should be cautious optimism to believe that these esters might serve as antisickling agents with some antibacterial action.

As already mentioned some hydroxybenzoic acids have been reported to possess antisickling activity, hence the need to modify them by esterification to increase their lipid solubility a factor pertinent to the penetration of cell membranes of which the erythrocyte membrane is one. In addition to this, acetonylation would result in activiated esters which are capable of both alkylation and acylation (Grundzins-

ski, 1958; Grundzinski, 1965), two independent pathways by which the HbS could be modified. In the later one it is being expected that acylation of the amino terminal of HbS would lead to an inhibition or reversal of sickling.

From the chemistry of hydantoins Lee (1976) formulated a reaction mechanism for the interaction of valine and methyl isocyanate (an antisickling agent). By analogy, the alkylation of the amino group of the N-terminal valine residues in beta-chains of HbS by an acetonyl ester might not end with the alkylamine derivative (I) but could proceed to form a cyclic product (II). Consequently, the following reaction mechanism is being postulated:

$$\begin{array}{c} \text{CH}_{3} \quad \text{O} \\ \text{CH}_{3} - \text{CH} - \text{CH} - \text{CH} - \text{CH} \\ \text{NH}_{2} \\ \text{CH}_{3} \\ \text{CH}_{3} \\ \text{CH}_{2} \\ \text{H+} \end{array} \xrightarrow{\text{CH}_{3}} \begin{array}{c} \text{CH}_{3} \\ \text{CH}_{2} \\ \text{CH}_{3} \\ \text{CH}_{3} \\ \text{CH}_{2} \\ \text{CH}_{3} \\ \text{CH}_{3} \\ \text{CH}_{2} \\ \text{CH}_{3} \\ \text{CH}_{4} \\ \text{CH}_{3} \\ \text{CH}_{4} \\ \text{CH}_{5} \\ \text{CH}_$$

Thus, the blocking of the beta-chain Val-1 residues may modulate the deoxy-oxy equilibrium of the sick-ling process (Arnone, 1972; Perutz, 1970).

This preliminary communication presents the synthesis and characterization of the following acetonyl esters (III-VI):

R.CO-O-CH2-COCH3 + NH(C2H5)|3 CI-

acetonyl esters triethylamine hydrochloride.

III. R=p-HO.C₆H₄ · IV. R=p-CH₃0C0C₆H₄

V. R=0-HO.C₆F - VI. R=C₆H₅CH₂

Work is in progress to screen the esters III-VI for antisickling action.

EXPERIMENTAL

Unless otherwise stated the following generalisations apply. The melting points (m.p.) were determined using Electrothermal m.p. apparatus and are uncorrected. Ultra-violet (U.V.) spectra were recorded for solutions in dichloromethane with the aid of either Unicam SP 8000 or Unicam SP 8-100 ultraviolet recording spectrophotometers. Molecular weights (M.W) were measured by mass spectrometry (M.S.9 instrument). Infra-red spectra (I.R.) were obtained using Unicam SP 1100 infrared spectrophotometer. Nuclear magnetic resonance spectra (N.M.R.) were determined on either Perkin-Elmer 60 MHz R-12A of EM-360 60 MHz spectrometer using tetramethylsilane as internal standard.

ACETONYL P-HYDROXYBENZOATE (III)

p-Hydroxybenzoic acid (6.9 g, 0.05 mole) was dissolved in 80 ml of ethyl acetate contained in a 250 m. round-bottomed flask. To the resultant solution were added 10.5 ml (0.08 mole) of anhydrous triethylamine (dried over KOH pellets) and 8.0 ml (0.1 mole) of chloroacetone, and the mixture was refluxed for 5 hours. The flask was then cooled and the contents filtered under reduced pressure from the precipitated triethylamine hydrochloride. The latter was washed with ethyl acetate and the washings combined with the filtrate. The filtrate, a dark brown liquid, was freed of ethyl acetate by evaporation under reduced pressure. The dark brown, viscous residue was left to stand at 30° in a fume cupboard.

The crude product (5.38 g) was recrystallised to constant m.p. from aqueous ethanol (25%). The pure ester III was a light yellow crystalline solid. It was soluble in ethanol, methanol, ether, slightly soluble in chloroform and apparently insoluble in water.

M.p. 118.5 – 1200.

U.V. spectrum showed max at 249 nm. I.R. spectrum; max (cm)-1): 3420 (phenolic OH, sharp and intense), 1730 (aliphatic ketone), 1700 (aryl ester), 1610 and 1600 (indicative of a C=C conjugated doubled bond in an aromatic ring), 1280 (C-O-C of an ester) and 855 (typical C-H out-of-plane bending vibration, showing a 1.4-disubstituted benzene ring). N. M.R. (8): 2.20 (CH₃, 3H, s), 4.82 (CH₂, 2H,s), 6.80 (2H, d, J=7.8 Hz) and 7.90 (2H, d, J=7.8 Hz) – aromatic protons. Total number of protons: 10, phenolic proton indistinguishable but confirmed by acetylation. M+ = 194.

ACETYLATION OF ESTER III (IV)

Ester III (0.5 g) was dissolved in pyridine (0.5 ml) and redistilled acetyl chloride (0.5 ml) was added dropwise, shaking after each addition. The resultant mixture was heated on a water-bath at 50-60° for 5 minutes. The mixture was cooled and poured into cold water (15 ml), sitrring vigorously until crystallization occured. A white, crystalline product with the characteristic sweet smell of esters was obtained on recrystallization from aqueous ethanol (50%). M.p. 100-102° I.R. spectrum showed no peak at around 3420 cm-1.

ACETONYL SALICYLATE (V)

The same procedure as for ester III was employed using twice the quantities of reagents. The crude yield was 20.6 g. Recrystallization from aqueous ethanol (50%) gave the pure ester V as a white, crystalline solid, m.p. 71-73°, soluble in ethanol, methanol, ether, chloroform, and apparently insoluble in water.

U.V.: λ max 239 nm and 310nm, I.R. max (cm-1) 3220 (phenolic OH lowered by strong intramolecular hydrogen bonding, fairly broad), 1730 (aliphatic ketone), 1680 (aryl ester carbonyl stretching, lowered by strong intramolecular hydrogen bonding), 1620 and 1590 (C=C conjugated double bond in an aromatic ring), 1255 (C-O-C of an ester), 760 (C-H out-of-plane deformation indicative of a 1,2-disubstituted benzene nucleus). N.M.R. (): 2.25 (CH₃, 3H, S) 4.90 (CH₂ + H, S) 7.90 (1H, m), 7.45 (1H, m) and 6.95 (2H, m) – aromatic protons; 10.40 (phenolic H, s). M+ = 194.

Ester V could not be acetylated under the conditions used for ester III.

ACETONYL PHENYLACETATE (VI)

The following reagents were used: 13.6 g (0.1 mole) of phenylacetic acid, 21 ml (0.16 mole) of trie thylamine and 16 ml of chloroacetone (0.2 mole).

The same reaction conditions as for ester III were applied. The residue obtained by evaporation of ethyl acetate under reduced pressure was extracted 5 times with ether. The ethereal extracts were evaporated to dryness and the residue was redissolved in ether. The solution was shaken subsequently with a saturated aqueous solution of NaHCo3, (to remove unreacted acid), and water. The ethereal layer was separated and dried over anhydrous MgSo4. The ether was distilled off and the residue fractionated to give the ester (VI) as a dark-brown liquid boiling over the range 120.5-1220 soluble in methanol, dichloromethane, ethanol, and very slightly soluble in water.

U.V.: max 236.5 nm and 261.5 nm I.R. max (cm -1): 1760-1730 (strong, fairly broad and likely to incorporate both the aliphatic ester and ketone groups), 1260 (C-0-C of an ester), 1380 (C-H vibra-

tion of acetyl group), 730 and 710 (monosubstituted benzene nucleus). N.M.R. (8): 7.20 (aromatic protons, 5H, s), 3.65 (-CH2CO2, 2H,S), 4.50 (O-CH2-CO, 2H, s) and 1.90 (COCH₃ 3H, s).

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