RESULTS AND DISCUSSION

The data in Table I show that each of the series III compounds behaved essentially identically toward each of the 10 test fungi. All of the series III compounds were inactive towards *Rhizopus stolonifer* and *Trichoderma viride* at all concentrations. In this regard they were inferior to the previously tested series IV compounds, which partially inhibited *R. stolonifer* at 10 μg/mL and *T. viride* at 100 μg/mL (15). With the exception of IIIe, the series III compounds partially inhibited the growth of the other eight test fungi at a concentration of 1 μg/mL. Employment of higher concentrations did not improve the antifungal activity. Compound IIIe differed from the other series III compounds only in that it was inactive towards *Chaetomium globosum* and *Cladosporium carpophilum* at 1 μg/mL. In comparison with the series III compounds, IVa was inactive towards *Aspergillus niger*, *Ch. globosum*, *Cl. carpophilum*, *Fusarium monoliforme*, *Penicillium notatum*, and *Saccharomyces cerevisiae* at 1 μg/mL. Compound IVb was also inferior to the series III compounds in that it was inactive towards *A. niger*, *Ch. globosum*, *F. monoliforme*, *Myrothecium verrucaria*, *P. notatum*, and *S. cerevisiae* at 1 μg/mL. Furthermore, the series IV compounds were inactive towards *S. cerevisiae* even at 10 μg/mL. Thus, the series III compounds generally exhibited higher antifungal activity than the series IV compounds.

The data in Table II show that all of the series III compounds were inactive towards the Gram-negative bacterium *Escherichia coli* at all concentrations. In this regard they resembled the previously tested IVa (15). All of the series III compounds behaved identically towards the two Gram-positive bacteria *Micrococcus agilis* and *Bacillus subtilis*, totally inhibiting the growth of these bacteria at a concentration of 1 μg/mL. In this regard they were somewhat better than IVa, which only partially inhibited *B. subtilis* at 1 μg/mL. The most effective series III compound against *Staphylococcus aureus* was IIIe, which totally inhibited the growth of this bacterium at 1 μg/mL. In contrast, IVa was inactive towards *St. aureus* at 1 μg/mL. Thus, the series III compounds generally exhibited higher antibacterial activity than IVa.

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Acylation of Hemoglobin by Aspirin-Like Diacyl Esters

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Abstract □ Aspirin-like diacyl esters of different steric disposition have been prepared and compared with acetylsalicylate in their abilities to modify hemoglobin.

Keyphrases □ Diacyl esters—aspirin derivatives, acylation of hemoglobin □ Hemoglobin—acylation by aspirin-like diacyl esters, sickle cell anemia

In a previous paper (1), it has been shown that aspirin can acylate hemoglobin and thereby provide a novel approach to the alteration of sickle hemoglobin. Acetylsalicylate is the prototype of a general class of monoacyl esters of salicylic acid. Consequently, a variety of such esters have been synthesized (2) and examined for their effectiveness in modifying hemoglobin S and in inhibiting the aggregation process leading to sickling.

Among variants in aspirin-like derivatives are the diacyl esters of dihydroxybenzoic acids. These seem attractive because they would present two active acyl groups to the protein when it forms a reaction complex with a reagent molecule. We have prepared, therefore, a series of such diacyl ester compounds of different steric structure and compared their abilities to modify hemoglobin.

EXPERIMENTAL SECTION

A variety of hydroxybenzoic acids were purchased from commercial sources[1]. Their mono- and diacyl esters were synthesized by methods described in the literature (3-7). The derivatives prepared are listed in Table I.

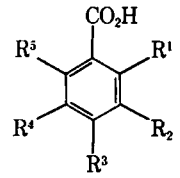
[1] Aldrich Chemical Co., for example.

Of the compounds obtained, only 3,5-diacetoxybenzoic acetic anhydride (XI) has not been reported in the literature. It appeared as a by-product in the mother liquor (chloroform–petroleum ether) from the preparation of the diacetoxybenzoic anhydride (X). The fine white precipitate which appeared in the mother liquor after standing for several hours was removed by filtration, washed with petroleum ether, and dried without heat, mp 71–72°C. This product, insoluble in aqueous $NaHCO_3$ at room temperature, bubbled on heating. It did not give a positive $FeCl_3$ test for free phenol. ^{1}H-NMR and

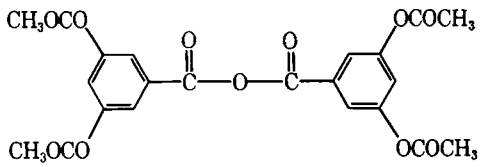
Table I—Modification of Hemoglobin by Aspirin-like Mono- and Diacyl Esters

Compound	Concentration, mM	Modification of Oxyhemoglobin A[a], %
I	10	22.5
II	10	23.1
	20	34.2
III	10	26.3
	20	36.8
IV	10	27.7
	20	37.3
V	5	32.3
	10	49.3
VI	5	19.1
	10	37.4
VII	10	78.7
VIII	5	36.3
	10	53.0
IX	5	23.8
	10	46.7
X	5	26.3
XI	5	45.0
	10	79.2

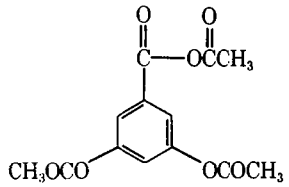
[a] Hemoglobin concentration = 1 mM.



I R^1=$OCOCH_3$, R^2=R^3=R^4=R^5=H
II R^3=$OCOCH_3$, R^1=R^2=R^4=R^5=H
III R^1=OH, R^3=$OCOCH_3$, R^2=R^4=R^5=H
IV R^1=$OCOCH_3$, R^3=OH, R^2=R^4=R^5=H
V R^1=R^3=$OCOCH_3$, R^2=R^4=R^5=H
VI R^1=R^4=$OCOCH_3$, R^2=R^3=R^5=H
VII R^1=R^5=$OCOCH_3$, R^2=R^3=R^4=H
VIII R^2=R^3=$OCOCH_3$, R^1=R^4=R^5=H
IX R^2=R^4=$OCOCH_3$, R^1=R^3=R^5=H



X



XI

microanalysis corresponded to expectations for XI. ^{1}H-NMR ($CDCl_3$-Me_4Si): δ 2.33 [d, 6, $(-O-COCH_3)_2$], 2.38 (s, 3, $-O-COCH_3$), and 7.2-7.75 ppm (m, 3, ArH).

Anal.—Calc. for $C_{13}H_{12}O_7$ (280.24): C, 55.72; H, 4.32. Found: C, 55.62; H, 4.17.

The preparation of hemoglobin and procedures for following its chemical modification by aspirin-like reagents have been described previously (2).

RESULTS AND DISCUSSION

In Table I, the efficacies of the diacyl esters in modifying hemoglobin are compared with those of corresponding monoacyl compounds. The following features are worthy of note.

Compound II, the *para* analogue of aspirin, is about as effective as I in acylating hemoglobin. Likewise the two positional monoacyl isomers of hydroxysalicylic acid, III and IV, are comparable in activity to each other and to aspirin (I). In contrast, the diacetoxy salicylic acid, V, is significantly more reactive, but the isomer VI is less so. By far the most effective of the diacyl hydroxysalicylic esters is VII, in which both acetyl groups are close to the anionic $-COO^-$ moiety. Alternative isomers of dihydroxybenzoic acids gave diacyl esters VIII and IX that did not compare in efficacy with VII, although VIII was significantly better than aspirin itself. Compound X, the anhydride of IX, was not better than aspirin, but the mixed anhydride XI was significantly better. In this series, therefore, the double *ortho* derivative VII is the most interesting compound and merits further investigation.

Previous studies (1, 8) have shown that aspirin-like reagents acylate sickle hemoglobin S just as extensively as they do hemoglobin A. Consequently effective compounds, such as 6-acetoxyacetylsalicylic acid (VII) of the present series, have potential as antisickling agents.

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