leg consists of a projection with two outer setae and a strong inner spine (Fig. 2f).

DISCUSSION

In Lang (1948) and Coull (1977) this species readily keys to Genus *Tisbe*. It differs from the described species of that genus in armature of the first leg exopod and the end of the first leg endopod, the rudimentary condition of the spine of the second leg exopod, and the armature of the mandibula exopod. It differs from *Tisbella pulchella* in the segmentation, shape, and armature of the first leg endopod, and presence of five, not four, setae on the terminal segment of the third leg endopod. A new genus might be described to accomodate this new species, but the close resemblance to species of *Tisbe* in segmentation and shape of the first leg endopod and in armature of other appendages make the separation of this new species from Genus *Tisbe* undesirable. The specific name honors this beautiful island and its friendly people.

It is of interest that males and female of *Tisbella pulchella* were collected from the same drainage ditch on Grand Cayman on 26 August 1978 and males and females of *Tisbe holothuriae* (Humes, 1957) in the same plankton hauls with the new species. The first legs of these species (fig. 2g, k), the second leg endopod, caudal rami, and fifth leg of the male *T. holothuriae* are shown here (Fig. 2h, j, i) to aid in distinguishing these two species from *T. caymanensis*. For detailed descriptions of these species see Wilson (1932), Yeatman (1963), and Humes (1957).

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ALIPHATIC ESTERS OF QUININE: SCREENING FOR ANTIPLASMODIAL ACTIVITY

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ABSTRACT

The C-9 aliphatic esters of quinine, formate through hexanoate, have been prepared. Interest in the effect that alkyl ester chains and chain length might have on antiplasmodial activity prompted an *in vitro* screening of these esters against two strains of *P. falciparum*. In the range of concentrations studied, the acetate and butanoate esters were inactive, while the activity of the propionate ester was comparable to that of quinine. Thus antimalarial activity is not related in an obvious way to the length of the alkyl group of the ester derivatives studied. Since the C-9 hydroxyl of quinine is required for antimalarial activity, an esterase enzyme must specifically catalyze the hydrolysis of the propionate ester to reveal the 9-OH group.

INTRODUCTION

In connection with research on the conformation of Cinchona alkaloids, we had occasion to prepare an homologous series of aliphatic esters *(1-6)* of the renowned antimalarial, quinine (Estensen et al., 1969), and became interested in a possible biological effect which alkyl ester chains and chain length might have on *in vitro* antiplasmodial activity. The present paper reports the results of this study.

METHODS

All thin-layer chromatograms were prepared using Eastman chromatogram sheets (silica gel, 0.2mm thickness). Plates were eluted with a solution of acetone and chloroform (1:1) and were visualized in an iodine-saturated chamber. Infared spectra were obtained on a Perkin-Elmer Model 710-B spectrophotometer. Nuclear magnetic resonance spectra were obtained on a JEOL Model JNM-PMX60 at 60 MHz. Chemical shifts are given in parts per million(δ) downfield from tetramethylsilane. Quinine hydrochloride used in the preparations is commercially available from Merck and Company, Inc.

Preparation of Quinine-O-Formate.

One ml of 97% formic acid was added dropwise to 2 ml of acetic anhydride at 0°C; warming at 40-50°C for 20 minutes and subsequent cooling produced formic acetic anhydride. Two ml of this solution was added dropwise over a period of 10 minutes with constant stirring to a cooled solution prepared from 0.995g of quinine hydrochloride dissolved in 5ml of pyridine. After cooling on ice for 20 minutes the mixture was decanted into 30ml of ethyl ether. Addition of petroleum ether deposited a thick oil (0.791g) which was taken up in 5 ml of benzene and washed with saturated $NaHCO_3$ solution. The organic layer was dried ($MgSO_4$), filtered, and evaporated to dryness to give 0.406g of a clear oil (quinine-O-formate). This sample was purified by column chromatography (silica gel, 2.5 × 1.5 cm) eluting with 1:1 chloroform: acetone and collecting 10 ml fractions. Fractions 4-9 yielded chromatographically pure formate ester: $R_f = 0.92$; IR(neat)-1720, 1620, 1590 cm^{-1} (no OH absorption). Quinine-O-formate was unstable upon normal storage and after a few days hydrolyzed (atmospheric moisture) to quinine.

Preparation of Quinine-O-Acetate and Quinine-O-Propionate.

Quinine hydrochloride (0.294g) in 6 ml of acetic anhydride was warmed for 3 mintes and left overnight at room temperature. The resulting solution was basicified with aqueous $NaHCO_3$ and extracted with $CHCl_3$; the organic layer was washed with water, dried over anhydrous $MgSO_4$, filtered and evaporated to dryness leaving 0.160g of crude acetate ester, $R_f = 0.27$. Recrystallization (ether/pentane) yielded 0.0765g of pure product: mp 115-117°C, lit 116-117°C (Harris, G., 1965); IR ($CHCl_3$) 1718, 1220, 1250 cm^{-1} (no OH absorption); NMR(CCl_4 δ 2.40(s); salicylate mp 180-182°C.

Quinine-O-propionate was prepared from propionic anhydride (5 ml) and quinine HCl-$2H_20$ (lg) in a procedure analogous to the preparation of acetate ester to yield 0.340g: mp 129-130°C, lit 129°C (Harris, G., 1965); IR ($CHCl_3$) 1730 cm^{-1}; salicylate mp 179-181°C.

Preparation of Quinine-O-Butanoate, Quinine-O-Pentanoate, and Quinine-O-Hexanoate.

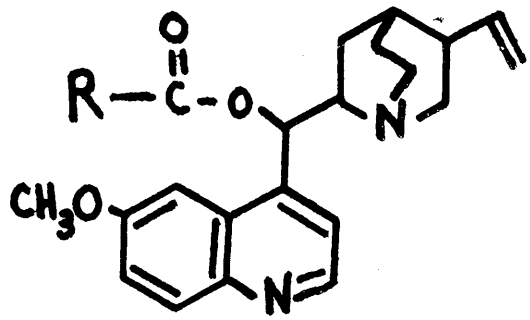
Butanoic acid (1.1ml) was refluxed with $SOCl_2$ (0.9ml) for 30 minutes in a round bottom flask equipped with a condenser and a $CaCl_2$ drying tube. Addition of 1g of quinine HCl·$2H_20$ resulted in a yellow, thick slurry which, after the addition of a few ml of anhydrous ether, was warmed 45 minutes and left at room temperature. The reaction mixture was taken up in $CHCl_3$ and the organic layer was washed with saturated $NaHCO_3$ (2 × 30 ml), dried ($MgSO_4$), filtered, and evaporated to dryness to give a dark orange oil (0.963g). The sample was purified by column chromatography (silica gel 2.5 × 1.5 cm) eluting with 1:1 chloroform; acetone, and collecting 20 ml fractions. Fractions 4-5 were taken up in benzene (25ml), washed with saturated $NaHCO_3$ and H_2O, dried ($MgSO_4$), filtered and evaporated to dryness leaving 0.255g of a clear thick oil (quinine-O- butanoate): $R_f = 0.42$; IR(neat) 1736 cm^{-1}; salicylate mp 195-196°C.

Quinine-O-pentanoate was prepared from pentanoyl chloride and quinine HCl·$2H_2O$ in a procedure analogous to the preparation of the butanoate ester to give 0.265g of a clear oil: $R_f = 0.24$; IR(neat) 1738 cm^{-1} (no OH absorption); salicylate mp 180-181°C.

Quinine-O-hexanoate was prepared from hexanoyl chloride and quinine HCl·$2H_2O$ in a procedure analogous to the preparation of the butanoate ester to give 0.424g of a colorless oil (quinine-O-hexanoate): $R_f = 0.18$; IR(neat) 1738 cm^{-1} (no OH absorption); salicylate mp 190-192°C.

Antiplasmodial Screenings.

An *in vitro* system was used in evaluating the drugs' activities against chloroquine resistant (Smith) and chloroquine sensitive (Camp) strains of *Plasmodium falciparum* (Sweeny, et al., 1982). Effectiveness was measured by comparing the uptake by parasite of a radioactively labeled nucleic acid precursor in the presence and absence of drug. Seven concentrations of the compounds were tested within the range of 1 to 100ug/ml and a plot was generated of radioactivity of the parasites as a function of drug concentration. An increase in drug activity associated with an increase in drug concentration was indicated by decreasing parasite radioactivity.



1: R=H
2: R=CH_3
3: R=CH_2CH_3
4: R=$CH_2CH_2CH_3$
5: R=$CH_2CH_2CH_2CH_3$
6: R=$CH_2CH_2CH_2CH_2CH_3$

Results and Discussion

Antimalarial screening (Sweeny, et al., 1982), as described in the Methods section, was attempted with all but the formate ester (*1*) which was unstable toward atmospheric moisture and hydrolyzed back to quinine shortly after its preparation. Of the compounds tested, the pentanoate (*5*) and hexanoate (*6*) esters could not be used since they were insoluble in the test medium. The activities of the remaining esters, acetate (*2*), propionate (*3*), and butanoate (*4*), were measured against two strains of *Plasmodium falciparum,* designated Camp and Smith. The compounds were screened at seven concentrations within the range of 1 to 100ug/ml. Of particular interest in the screening of the aliphatic esters were their levels of antimalarial activity relative to quinine, an intercalator of the DNA helix (Hahn, 1969).

In the screening of quinine and its derivatives, levels of activity were measured by comparing uptake by the parasite of a radioactively labeled nucleic acid precursor in the presence and absence of drug. Against the Camp strain, absorption of the radioactively labeled nucleic acid precursor was reduced by ½ at a quinine concentration of 36ug/ml and at a propionate ester (*3*) concentration of 85ug/ml. The acetate (*2*) and butanoate (*4*) esters, however, showed no measurable levels of activity in the range of concentrations studied. Against the Smith strain, absorption was reduced by ½ at a concentration of 80ug/ml of quinine while a concentration of 61ug/ml of the propionate ester was as effective; no measurable levels of activity were seen for either the acetate (*2*) or butanoate (*4*) esters.

The results obtained in testing against the two *Plasmodium* strains seem consistent. Against both strains, quinine and its propionate ester (*3*) are active, while neither the acetate (*2*) nor the butanoate (*4*) esters show any detectable levels of activity. These results indicate that antimalarial activity is not related in an obvious way to the length of the alkyl group of the ester derivatives studied.

Previous research of quinine derivatives as antimalarials has demonstrated that substitution by Cl or H at the C-9 hydroxyl group increases the compound's toxicity and abolishes its antiplasmodial activity (de Jongh, et al., 1954); thus the intact OH is essential for activity (Goodman, et al., 1970). In this regard, differences in the levels of activity of the esters are most likely due to a specific enzyme which catalyzes the hydrolysis of the propionate ester (*3*) to reveal the 9-OH group and quinine itself. The acetate (*2*) and butanoate (*4*) esters are inactive within the range of concentrations studied and thus are apparently not hydrolyzed to yield the active drug. Since quinine itself exhibits tissue irritant action whether taken orally or by injection (Goodman, et al., 1970), the results described in this paper imply a potential of quinine-O-propionate as a less harmful "pro-drug" in antimalarial therapy.