phenothiazine ring in 6 may obstruct the attachment of 6 to appropriate receptor sites and thus lead to an inactive molecule. The disappointing results with 6 and 9 discouraged us from further endeavors in this area.

Experimental Section

Melting points are uncorrected; all compounds were analyzed for C, H, and N and gave results within ±0.4% of the theoretical values. Ir and nmr spectral data were consistent with the structures assigned.

10-[3-(4-Methylpiperazinylpropyl)]-2,2′-bis(trifluoromethyl)-3,10′-biphenothiazine (Tentatively 6). To a solution of 1 (5.35 g, 20 mmol) in dry benzene (25 ml) at 50° was added sodamide (0.8 g, 21 mmol) and the mixture stirred at 50° for 1 hr. 1-(3-Chloropropyl)-4-methylpiperazine (3.9 g, 22 mmol) (prepared according to Marxer[7]) was now introduced and the mixture stirred under reflux overnight and filtered. The filtrate was freed from benzene *in vacuo* and the residue triturated with ether (50 ml). The resultant solid was filtered off and washed with ether to give 6 (2.3 g), mp 225–227°. An additional quantity was recovered from the ether filtrate: total yield 2.7 g (40%). Biphenothiazine 6 was recrystallized from the acetone–alcohol mixture: mp 227–228°; *m/e* 672 (M^+), 572, 531, 266. *Anal.* ($C_{34}H_{30}F_6N_4S_2$) C, H, N. The maleate of biphenothiazine 6 (from MeOH) had mp 209–211°. *Anal.* ($C_{38}H_{34}F_6N_4O_4S_2$) C, H, N. From the ether mother liquors, 5 was isolated through dilute HCl and purified by distillation at 180–200° (0.02 mm); *m/e* 407 (M^+), 306, 266. Trifluoperazine (5) was characterized as the HCl salt: mp 247–249° (from EtOH). *Anal.* ($C_{21}H_{26}F_3Cl_2N_3S$) C, H, N.

10-(2-Dimethylaminoethyl)-2,2′-bis(trifluoromethyl)-3,10′-biphenothiazine (Tentatively 9). Phenothiazine 1 (4 g, 15 mmol) was treated first with sodium hydride (0.8 g of 50% suspension in mineral oil, 17 mmol) in dioxane at 50° for 0.5 hr and then with dimethylaminoethyl chloride (1.6 g, 15 mmol) overnight at 70°. The mixture was filtered and the filtrate stripped of solvent *in vacuo*. The residue was partitioned between dilute HCl and ether. The acid layer was separated, made basic, and extracted with ether. The ether layer was separated and evaporated and the residue triturated with ether. The insoluble product was filtered off and crystallized from ether–hexane to give 9 (0.9 g, 20%): mp 174°; *m/e* 603 (M^+), 531, 266. *Anal.* ($C_{30}H_{23}F_6N_3S_2$) C, H, N.

Likewise was prepared 10-methyl-2,2′-bis(trifluoromethyl)-3,10′-biphenothiazine (tentatively 8) in 20% yield: mp 230–232° (from MeOH); M^+ at *m/e* 546. *Anal.* ($C_{27}H_{16}F_6N_2S_2$) C, H, N.

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Microbial Hydroxylation of Acronycine

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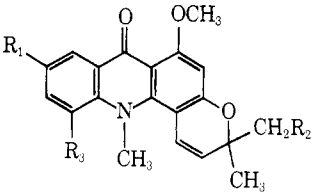
Acronycine (1) is an acridone alkaloid isolated from the bark of *Acronychia bauri* Schott.[1] It possesses broad-spectrum antitumor activity against experimental neoplasms in laboratory animals[2] and is now being clinically evaluated.

Sullivan, *et al.*,[3] have studied the metabolism of acronycine in animals. A number of metabolites were detected in body fluids. However, insignificant amounts of these metabolites were obtained to determine their antitumor activity. The following is a report of the production of two of these metabolites by microbial conversion.



1, R_1, R_2, R_3 = H
2, R_1 = OH; R_2, R_3 = H
3, R_1, R_3 = H; R_2 = OH
4, R_1 = H; R_2, R_3 = OH

Sullivan, *et al.*,[3] observed that hydroxylation was the major route of metabolism of acronycine in laboratory animals and in man. In particular, the hydroxylation of acronycine at C-9, the position para to the acridone nitrogen, occurred in five species.

Addition of acronycine to a metabolizing culture of *Aspergillus alleaceus* produced 9-hydroxyacronycine (2). The microbial hydroxylation of alkaloids frequently occurs para to an aromatic amine, *i.e.*, hydroxylation of tryptophan,[4] yohimbine,[5] and *Vinca* alkaloids.†

Addition of acronycine to a metabolizing culture of *Streptomyces spectabilicus* resulted in the hydroxylation of one of the C-3 methyl groups to give 3. This metabolite was not observed in body fluids of man. However, compound 4, which is possibly a metabolite of 3, was observed. The results of this work indicate that metabolism patterns exhibited by animals might be expected to occur in certain microorganisms.

Compounds 2 and 3 were both tested for antitumor and antiviral activity. Neither compound was active in the herpes virus test that is known to be sensitive to acronycine. Similarily, neither compound produced tumor inhibition or life prolongation in mice implanted with X5563 plasma cell myeloma[2] or C-1498 myelogenous leukemia.[2] Animals treated with the same level of acronycine showed a positive response in the test systems.

These data suggest that the antitumor activity of acronycine is not due to 3. Indeed, hydroxylation may represent removal of the active compound from the animal *via* glucuronide formation.

Experimental Section

Screening Procedure. Acronycine, 250 μg/ml, was added to erlenmeyer flasks containing 100 ml of 48-hr cultures. At daily intervals for 4 days samples were withdrawn from the flasks and extracted with an equal volume of $CHCl_3$. The extracts were chromatographed on Merck silica gel thin-layer plates. The chromatograms were developed in EtOAc–MeOH–diethylamine (85:10:5) and observed under uv light at 366 mμ. Approximately 500 cultures, some of known identity and some soil isolates, were screened.

Preparation of 9-Hydroxyacronycine (2). *A. alleaceus*, QM 1915 (25 l.), was grown at 25° in a 40-l. fermentor containing the following media constituents (g/l.): corn meal 25, sucrose 10, yeast extract (Difco) 5, soytone (Difco) 10, $MgSO_4 \cdot 7H_2O$ 2, KCl 3, and K_2HPO_4 2. Acronycine, 2 g in 50 ml of ethanol, was added in equal portions to the fermentor at 66, 74, and 90 hr after inoculation. After incubation for an additional 72 hr after the last substrate addition, the resulting cells and broth were separated by

†Unpublished results of these laboratories.

filtration. The $CHCl_3$ extract of the broth showed no acronycine metabolites when chromatographed on thin-layer plates. The cells were extracted with two 3-l. portions of MeOH and the methanolic extracts were combined and concentrated to give 7.39 g of a yellow powdery precipitate. Tlc indicated that the precipitate was a mixture of **1** (R_f 0.55) and **2** (R_f 0.22). A sample of this mixture was treated with ethereal diazomethane to convert **2** to the corresponding methyl ether. Vapor-phase chromatography (hydrogen flame, 270°, 4-ft glass column packed with 3.8% W-98 methylvinyl silicone gum on 80–100 mesh Diatoport S) of this mixture indicated an approximate 1:4 ratio of **1** and the methyl ether derivative of **2**. Silica gel (Grace 62) column chromatography of the methanol precipitate failed to remove the last traces of **1** from **2**. However, compound **1** proved to be slightly more soluble than **2** in hot MeOH. Repeated crystallization of column preparations of **2** finally gave **2** free of **1**, as shown by vpc of its methyl ether derivative: yield 4.8 g. The material was identical in all respects with that reported for 9-hydroxyacronycine. *Anal.* ($C_{20}H_{19}O_4N$) C, H, O.

Preparation of 3-Hydroxymethylacronycine (3). A total of 3 g of acronycine, 100 μg/ml, was added to erlenmeyer flasks containing 100 ml of a 48-hr culture of *Strept. spectabilicus*, NRRL 2494. The culture was grown at 30° in a medium containing (g/l.): cerelose 25, corn starch 10, peptone (Wilson) 10, NA-Amine A (Sheffield) 4, molasses (Black Strap) 5, $MgSO_4 \cdot 7H_2O$ 0.5, $CaCO_3$ 2. After incubation for an additional 72 hr, the resulting cells and broth were separated by filtration. The cells were extracted with MeOH and the extract was concentrated by vacuum to an aqueous solution. This solution was added to the filtered broth and the combined solution was extracted (volume/volume) with $CHCl_3$. The $CHCl_3$ extract was concentrated by vacuum to give 6.53 g of noncrystalline material which was chromatographed on a column of 500 g of Grace 62 silica gel packed in benzene. The column was eluted with benzene and benzene–EtOAc mixtures. Elution with benzene–EtOAc (9:1) gave 460 mg of acronycine. Elution with benzene–EtOAc (2:3) gave 930 mg of a material which showed one spot on tlc, R_f 0.38. Recrystallization from $CHCl_3$–hexane gave 430 mg of crystalline **3**. The mass spectrum, vpc retention time, and infrared spectrum of this material were identical with those of 3-hydroxymethylacronycine.[3] *Anal.* ($C_{20}H_{19}O_4N$) C, H, O.

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4- (and 5-) (2-Thienyl)-1-naphthaleneacetic Acids as Antiinflammatory Agents

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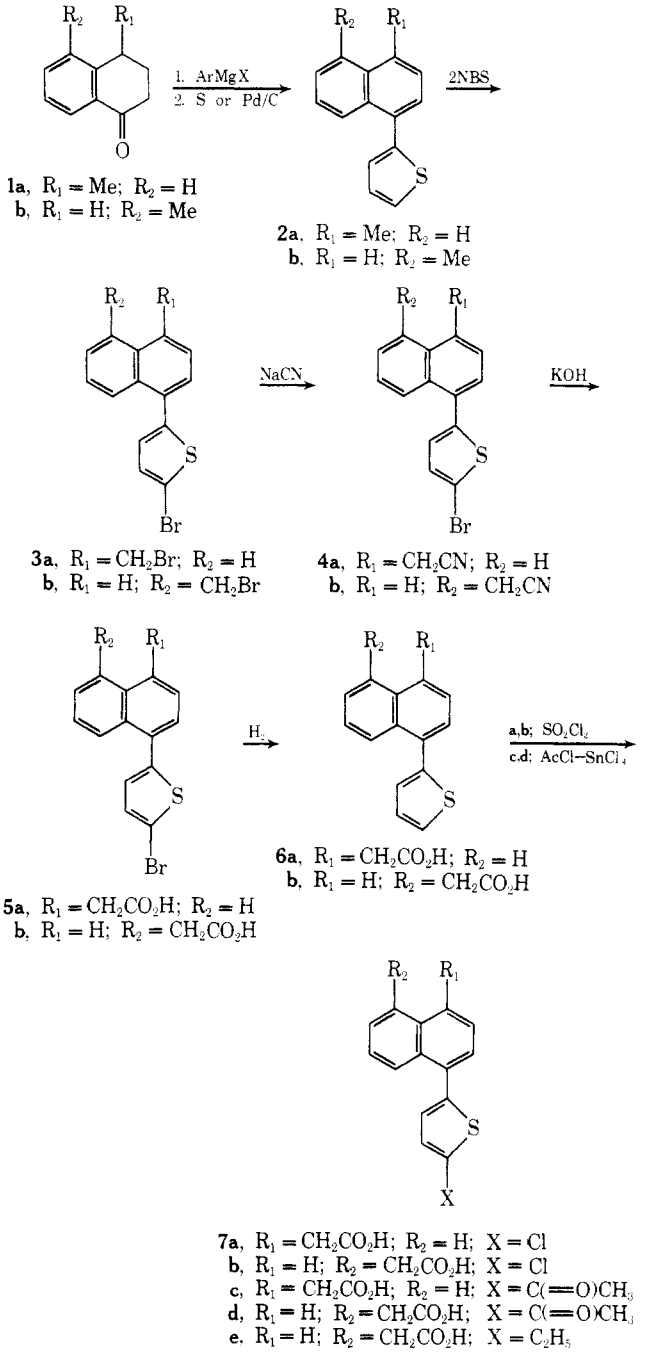
In an earlier paper[1] we described a series of substituted 4- and 5-phenyl-1-naphthaleneacetic acids which showed high potency as antiinflammatory agents. In the present paper we describe a series of 4- and 5-(2-thienyl)-1-naphthaleneacetic acids.[2] Measured by the anti-uv-erythema test in guinea pigs, several members of this series exhibit high potency as antiinflammatory agents.

Chemistry. The compounds were prepared as outlined in Scheme I starting with 3,4-dihydro-4- (or 5-) methyl-1(2*H*)-naphthalenone (**1**). In general, the intermediates **1–4** were not extensively purified but were carried through the sequence to the acetic acid stage where acidic product was easily separated. The crude acids were then purified by chromatography on silica gel. Most of the neutral material carried along in the reaction sequence arises from the fact that the side-chain bromination frequently does not go to completion, and the unreacted methyl derivative is thus carried along in subsequent reactions.

Scheme I



1a, R_1 = Me; R_2 = H
b, R_1 = H; R_2 = Me

2a, R_1 = Me; R_2 = H
b, R_1 = H; R_2 = Me

3a, $R_1 = CH_2Br$; R_2 = H
b, R_1 = H; $R_2 = CH_2Br$

4a, $R_1 = CH_2CN$; R_2 = H
b, R_1 = H; $R_2 = CH_2CN$

5a, $R_1 = CH_2CO_2H$; R_2 = H
b, R_1 = H; $R_2 = CH_2CO_2H$

6a, $R_1 = CH_2CO_2H$; R_2 = H
b, R_1 = H; $R_2 = CH_2CO_2H$

7a, $R_1 = CH_2CO_2H$; R_2 = H; X = Cl
b, R_1 = H; $R_2 = CH_2CO_2H$; X = Cl
c, $R_1 = CH_2CO_2H$; R_2 = H; X = C(=O)CH_3
d, R_1 = H; $R_2 = CH_2CO_2H$; X = C(=O)CH_3
e, R_1 = H; $R_2 = CH_2CO_2H$; X = C_2H_5

Aromatization of the intermediate dihydro derivative to give **2** can be accomplished by heating with sulfur or, less conveniently, by heating with Pd/C in the presence of nitrobenzene. In the case of **2a**, this aromatization is easily followed with nmr by the disappearance of the aliphatic methyl doublet centered at 1.3 ppm in the dihydro derivative and the appearance of the aromatic methyl singlet at 2.7 ppm. With **2b**, the methyl group in the dihydro compound is on a phenyl ring and shows a singlet at 2.18 ppm. The completely aromatized molecule has the methyl