Notes

10-Alkyl- and 10-Aminoalkyl-2,2′-bis(trifluoromethyl)-3,10′-biphenothiazines†

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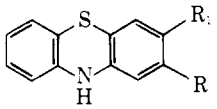
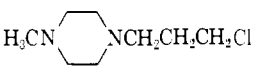
We wish to report the formation of the novel title compounds which we have encountered for the first time during an attempt to prepare trifluoperazine, 10-[3-(4-methyl-1-piperazinylpropyl)]-2-trifluoromethylphenothiazine (**5**),[1] by a published procedure[2] involving the preparation of the sodium salt of 2-trifluoromethylphenothiazine (**1**) in benzene using sodamide and heating the salt under reflux with 1-(3-chloropropyl)-4-methylpiperazine (**4**) overnight, without any precaution to exclude air. Work-up gave as the ether-insoluble part the aminoalkylated biphenothiazine derivative presumably **6**, mp 227–228° in 40% yield, and as the ether-soluble part, the normal product **5** in 58% yield as an oil. The gross structure of **6** was consistent with the analytical and mass spectral data. This dimer, presumably **6**, had no NH band in the ir spectrum. The attachment of the two phenothiazine moieties in the dimer as shown in **6** is tentative and is based on its nmr spectrum. Thus, in the 220-MHz spectrum of **6** in $CDCl_3$, a singlet (1 H) and a complex multiplet (1 H) were seen in the aromatic region at δ 6.42 and 6.20 ppm separated significantly from the signals of the other aromatic protons which were all downfield. The two high-field signals were absent in the 220-MHz nmr spectra of **1** and **5**. It is reasonable to assign the singlet to the proton at C-4 and the multiplet to the proton at C-9′, ascribing their high-field location to the mutual shielding influence of the two phenothiazine moieties which must be noncoplanar. The proton at C-1′ must also experience this shielding, but probably the deshielding influence of the adjacent CF_3 group at C-2′ annuls the other effect.



1, R = CF_3; R_1 = H
2, R = H; R_1 = Cl
3, R = R_1 = H



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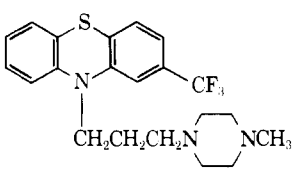

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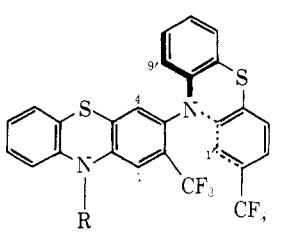

6, R = $CH_2CH_2CH_2N$⟨⟩NCH_3
7, R = H
8, R = CH_3
9, R = $CH_2CH_2N(CH_3)_2$

Biphenothiazine, presumably **6**, was formed in appreciable amounts also when the reaction was conducted in dioxane, using sodium hydride (15% yield) or sodamide (40% yield) and the halide **4**, but only negligibly when a nitrogen stream was used. In parallel experiments, a solution of **1** in dioxane or benzene was stirred at 50–60° with sodium hydride or sodamide without using a nitrogen atmosphere. The recovered products were shown to contain significant quantities of the dimer, presumably **7**, by means of nmr and mass spectral data. The presumed dimer **7** could not be isolated pure but upon alkylation with chloride **4** again gave **6** in high yield. Phenothiazine **1** was unaffected by heating in dioxane alone, without sodium hydride; so was **5** when heated with the sodium salt of **1** in dioxane. It thus appears that the anion of **1** goes over to a radical which dimerizes oxidatively to **7** which in turn gets alkylated to **6**. Treatment of **1** with sodium hydride in DMF followed by chloride **4** resulted only in recovery of **1**. Neither **5** nor **6** was formed.

Treatment of **1** with sodium hydride in dioxane followed by reaction of the sodium salt with methyl iodide or dimethylaminoethyl chloride gave phenothiazines, presumably **8** and **9**, in about 20% yield, along with the normal products.

3-Chlorophenothiazine (**2**) upon treatment with sodium hydride in dioxane appeared to form a 10,10′-biphenothiazine, mp >300° (M^+ at *m/e* 464, 466, 468). The product has no NH band in the ir spectrum and was not converted to an aminoalkyl derivative by **4**. Phenothiazine (**3**) itself after reaction with sodium hydride, followed by treatment with halide **4**, yielded a poorly characterized product, whose mass spectrum indicated that even a trimeric species with the *N*-methylpiperazinylpropyl side chain (*m/e* 733) was present in addition to a dimer (*m/e* 536).

The formation of biphenothiazines from phenothiazines has been reported in recent years to occur in the reaction of phenothiazine perchlorate with fluoride systems[3] and during oxidation of phenothiazines with iodine in DMSO,[4] but to our knowledge, ours is the first observation of this phenomenon under normal alkylating conditions used for the preparation of the well-known tranquilizers of the phenothiazine group.[5]

Behavioral and neurological changes induced in CF male mice by **6** (as maleate) and **9**, given in graded doses up to 500 mg/kg po and 125 mg/kg ip, were examined by a multidimensional test system according to Morpurgo.[6] The compounds were also examined for CNS effects in albino rats at 100 mg/kg given orally as well as intraperitonially. No significant behavioral or neurological changes were observed at these doses in mice or rats. Both compounds did not possess anticonvulsant activity and were ineffective up to 30 mg/kg ip in protecting mice against seizures induced by maximal electroshock or by strychnine and pentylenetetrazole. They did not antagonize reserpine-induced ptosis, tremorine-induced tremors and salivation, mescaline-induced stereotypy, or amphetamine-induced hyperthermia in mice.

In contrast, trifluoperazine (**5**) produced a picture of dose-dependent CNS depression when administered to mice in a dose range of 25–400 mg/kg po or 5–100 mg/kg ip. Marked sedation, ptosis, hypoactivity, ataxia, and muscle relaxation were present, while cataleptic symptoms occurred at all doses and lasted for almost 24 hr. Although **5** did not protect mice against electrically or chemically induced seizures up to 30 mg/kg ip and did not antagonize reserpine-induced ptosis in mice, it inhibited at this dose tremors induced by amphetamine. Trifluoperazine (**5**) also antagonized CNS stimulation and stereotypy induced by mescaline at 2–4 mg/kg po in mice.

It is possible that compared to **5**, the extra orthogonal

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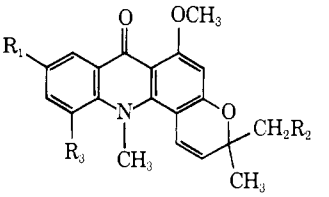
Acronycine (**1**) is an acridone alkaloid isolated from the bark of *Acronychia bauri* Schott.[1] It possesses broad-spectrum antitumor activity against experimental neoplasms in laboratory animals[2] and is now being clinically evaluated.

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.