following administration of isoprenaline. A control group of five animals received iv saline and was treated in the same manner as drug-treated groups. Statistical analysis was performed as described above.

Registry No. **3a**, 101479-72-5; **3a** (oxalate), 114652-88-9; **3b**, 101479-70-3; **3b** (oxalate), 114652-89-0; **3c**, 114652-90-3; **3c** (oxalate), 114652-91-4; **3d**, 114652-92-5; **3d** (oxalate), 114652-93-6; **3f**, 113296-04-1; **3f** (oxalate), 114652-94-7; **3g**, 114652-95-8; **3g** (oxalate), 114652-96-9; **5**, 156-38-7; **6**, 17138-28-2; **7**, 114652-97-0; **8**, 114652-98-1; **9a**, 114652-99-2; **9b**, 114653-00-8; **9c**, 114653-01-9; **9d**, 114653-02-0; **9e**, 114653-03-1; **9f**, 114653-04-2; **10a**, 114653-05-3; **10b**, 114653-06-4; **10c**, 114653-07-5; **10d**, 114653-08-6; **10e**, 114653-09-7; **10f**, 114653-10-0; **11a**, 114653-11-1; **11b**, 114653-12-2; **11c**, 114653-13-3; **11d**, 114653-14-4; **11f**, 114653-15-5; **11g**, 114653-16-6; **12**, 6975-91-3; **13**, 83053-47-8; **14**, 114653-17-7; adamantylmethanol, 770-71-8; adamantylethanol, 6240-11-5; (+)-isopinocampheol, 24041-60-9; 1*s*-*endo*-borneol, 464-45-9; *exo*-norborneol, 497-37-0; *endo*-norborneol, 497-36-9; cyclohexanol, 108-93-0; chloroacetyl chloride, 79-04-9.

Effects of Sulfur-Containing Analogues of Stearic Acid on Growth and Fatty Acid Biosynthesis in the Protozoan *Crithidia fasciculata*

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A variety of analogues of stearic acid in which one of the methylene groups was replaced by a sulfur atom were examined as inhibitors of growth and fatty acid biosynthesis in the trypanosomatid protozoan *Crithidia fasciculata*. The 8-, 9-, 10-, and 11-thiastearic acids were found to suppress the synthesis of the cyclopropane-containing fatty acid dihydrosterculic acid (9,10-methyleneoctadecanoic acid) at micromolar concentrations in the growth medium, and all but the 9-thiastearate were found to inhibit the growth of the protozoa at comparable concentrations. The most potent inhibitor, 8-thiastearic acid (I_{50} for growth = 0.8 μM; I_{50} dihydrosterculate synthesis = 0.4 μM), was also observed to inhibit the synthesis of γ-linolenic acid at a similar concentration. The sulfoxide derivatives of the 9- and 10-thiastearates were found to have little effect on growth or fatty acid synthesis, and several long-chain amides of 3-amino-1,2-propanediol were found to have effects similar to those of the fatty acids from which they were derived.

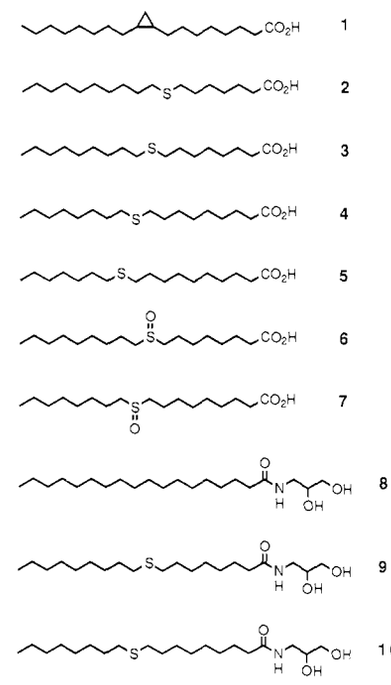
Dihydrosterculic acid (9,10-methyleneoctadecanoic acid, **1**), a cyclopropane fatty acid widely distributed in bacteria and plants,[1] is also a major constituent of the phospholipids of many trypanosomatid flagellates, including several pathogenic species of *Leishmania*.[2,3] The presence of compound **1** is one of the "biochemical peculiarities" of these protozoa[4]—at least with respect to mammals, which do not synthesize or require cyclopropane fatty acids. If dihydrosterculic acid is not merely present in the protozoa but in fact *required* for normal growth, then specific inhibitors of the biosynthesis of **1** might be useful antiparasitic agents.

We recently observed that 10-thiastearic acid (**4**, Chart I) is a potent inhibitor of dihydrosterculic acid biosynthesis in *Crithidia fasciculata*.[5] In addition, we found that the growth of the protozoa is inhibited at concentrations comparable to those required for the inhibition of cyclopropane synthesis, suggesting that dihydrosterculic acid is required for the normal growth of these organisms. In this paper we report additional studies with 10-thiastearic acid and examine the effects of a variety of other sulfur-containing analogues of stearic acid on growth and fatty acid biosynthesis in *C. fasciculata*.

Chemistry

The syntheses of the free fatty acids in which C-9 or C-10 is replaced by a sulfur-containing functional group

Chart I



(**3**, **4**, **6**, and **7**) were described previously,[6] and the 8- and 11-substituted acids (**2** and **5**) were prepared by using similar methodology.

The syntheses of the long-chain amides **8**–**10** are somewhat unusual. We first attempted to prepare these *N*-(2,3-dihydroxypropyl) amides by condensation of the appropriate fatty acyl chlorides with 3-amino-1,2-

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(2) Meyer, H.; Holz, G. G., Jr. *J. Biol. Chem.* **1966**, *241*, 5000–5007.
(3) (a) Fish, W. R.; Holz, G. G., Jr.; Beach, D. H.; Owen, E.; Anekwe, G. E. *Mol. Biochem. Parasitol.* **1981**, *3*, 103–115. (b) Holz, G. G., Jr. In *Leishmaniasis*; Chang, K.-P., Bray, R. S., Eds.; Elsevier: New York, 1985.
(4) Opperdoes, F. R. *Br. Med. Bull.* **1985**, *41*, 130–136.
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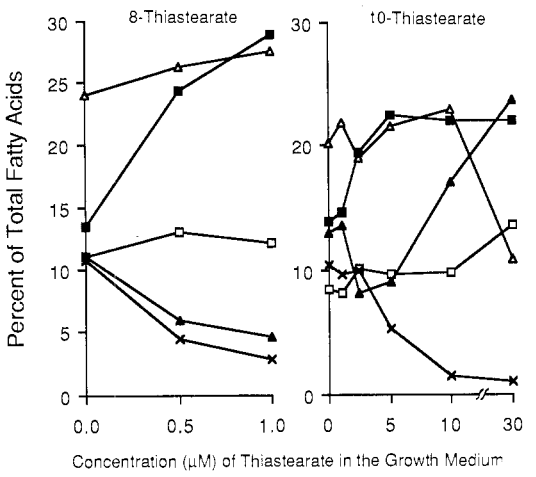

Figure 1. The effects of various concentrations of 8-thiastearic acid (**2**) (left) and 10-thiastearic acid (**4**) (right) on the synthesis of C-18 and C-19 fatty acids in *C. fasciculata*. Results: (□) stearic acid, (■) oleic acid, (△) linoleic acid, (▲) γ-linolenic acid, (X) dihydrosterculic acid.

propanediol in the presence and absence of various bases; all such reactions were unsuccessful. Aqueous workups were employed, and only free fatty acids were isolated from the reaction mixtures. Subsequently it was found that the simple procedure of heating the free acid with a slight excess of the amino diol in the absence of solvent at 190 °C gave satisfactory yields of the desired amides.

Biological Results and Discussion

Effects of Thioether-Containing Fatty Acids. Our initial objective in these studies was to design and prepare a compound that would specifically inhibit cyclopropane fatty acid biosynthesis in parasitic protozoa. Inasmuch as dihydrosterculate synthesis involves the formation of new carbon–carbon bonds to C-9 and C-10 of oleate, 9- and 10-substituted fatty acids were the most obvious candidates for inhibitors of cyclopropane synthesis, and we have outlined previously[5] a possible mechanism for suppression of this process by a sulfur-substituted fatty acid. The sulfur atom in a thioether is generally regarded to have steric requirements that are very similar to those of a methylene group; therefore, it was not too much to expect that thioether-containing fatty acids would be processed in a normal fashion by the protozoa except when enzymatic reactions are attempted near the sulfur site itself. Two such reactions were of concern to us: (a) the 9,10-dehydrogenation (desaturation) of stearate to give oleate and (b) the 9,10-methylenation of oleate to give dihydrosterculate. It is preferable not to interfere with the desaturation event, since mammals as well as protozoa require oleate. Fortunately, we knew that 10-thiastearyl coenzyme A is an excellent substrate, but not an inhibitor, for mammalian stearyl-CoA desaturase,[8] so 10-thiastearic acid (**4**) was chosen as the first compound to be examined for its effects on cyclopropane fatty acid synthesis in *C. fasciculata*.

The effects of various concentrations of 10-thiastearic acid on the production of C-18 and C-19 fatty acids by *C. fasciculata* in culture are illustrated in Figure 1. At a concentration of 10 μM **4**, the amount of dihydrosterculic acid (**1**) formed (as the percent of total fatty acids) was reduced by 1 order of magnitude, and the amount of oleic acid present (the precursor of **1**) was increased correspondingly. The amounts of stearic, linoleic, and γ-linolenic acids in the control (0 μM) and 10 μM cultures were similar.[9] At even higher concentrations of **4** (30 μM), the amount of γ-linolenate formed increases dramatically, and linoleate is similarly decreased. γ-Linolenic acid presumably is formed from linoleic acid, so this latter effect does not represent an inhibition of fatty acid synthesis by **4**; perhaps it is a response to dihydrosterculate deprivation.

Table I. Approximate Concentrations (μM) of Fatty Acid Analogues Required for 50% Inhibition (I_{50}) of Growth and Dihydrosterculate Synthesis in *C. fasciculata*

analogue	growth	dihydrosterculate synthesis
stearic acid	*a*	*b*
8-thiastearic acid (**2**)	0.8 ± 0.4	0.4 ± 0.1
9-thiastearic acid (**3**)	>100	0.9 ± 0.3
10-thiastearic acid (**4**)	10 ± 2	4 ± 1
11-thiastearic acid (**5**)	3 ± 2	3 ± 1
8-(nonylsulfinyl)octanoic acid (**6**)	>100	*b*
9-(octylsulfinyl)nonanoic acid (**7**)	>100	*b*
N-(2,3-dihydroxypropyl)octadecanamide (**8**)	*b*	*b*
N-(2,3-dihydroxypropyl)-8-(nonylthio)-octanamide (**9**)	>100	8 ± 3
N-(2,3-dihydroxypropyl)-9-(octylthio)-nonanamide (**10**)	17 ± 4	12 ± 5

[a] 10–20% stimulation of growth at 30–100 μM. [b] No inhibition observed.

10-Thiastearate also inhibits the growth of *C. fasciculata*. A 50% inhibition of growth was observed when **4** was present at 10 μM in the medium; 50% inhibition of dihydrosterculate synthesis occured at ca. 4 μM **4** (see Table I). The similarity of the concentrations required for the two kinds of inhibition suggested that **1** is required for normal growth of *C. fasciculata*. Added fatty acids are not in general inhibitory; for example, when stearic acid is added to the medium, protozoan growth is *stimulated*, and the intracellular fatty acid composition is unchanged. On the other hand, addition of **1** to the growth medium does not reverse the effects of 10-thiastearate, but it is unknown if added **1** is transported into the cells and incorporated into the phospholipids.

The success of **4** as an inhibitor of growth and dihydrosterculate synthesis led us to examine other thioether-containing analogues of stearic acid for their effects in *C. fasciculata*. The 8-, 9-, and 11-thiastearic acids (**2**, **3**, and **5**, respectively) were prepared and tested, and the results are summarized in Table I. All four thiastearates produced a 50% inhibition of the synthesis of **1** at comparable concentrations in the growth medium, ranging from 0.4 to 4 μM. However, the effects of these compounds on protozoan growth were surprisingly varied. 8-Thiastearic acid was a very powerful growth inhibitor (I_{50} = 0.8 μM); essentially no protozoan growth was observed when the medium contained >3 μM **2**. In contrast, 9-thiastearic acid was only a modest growth inhibitor (I_{50} >100 μM).

The concentrations of 8-, 10-, and 11-thiastearate required for 50% inhibition of growth and 50% inhibition of dihydrosterculate synthesis were very similar, consistent with the suggestion that dihydrosterculate is an essential metabolite in *Crithidia*. However, 9-thiastearic acid (**3**) was at least 100 times as potent an inhibitor of cyclopropane synthesis as of growth. Therefore, either the suppression of dihydrosterculate biosynthesis alone is insufficient to halt growth or compound **3**, while inhibiting

(8) Strittmatter, P.; Ziering, D.; Pascal, R. A., Jr., unpublished results.

(9) It should be noted that *C. fasciculata* produces γ-linolenic acid but very little if any α-linolenic acid (ref 2).

the synthesis of 1, may substitute for 1 in some essential function(s). The former, simpler explanation seems more likely, but we are unable to resolve this issue at this time.

The effects of the 9-, 10-, and 11-thiastearates on the fatty acid distribution in *C. fasciculata* are rather similar, though the concentration dependencies vary. However, 8-thiastearic acid (2), the most potent growth inhibitor, differs from the others in one important respect: in addition to inhibiting dihydrosterculate synthesis, 2 also inhibits γ-linolenate synthesis at comparable concentrations (see Figure 1). At a concentration of 1.0 μM 2 in the growth medium, the amount of dihydrosterculate and γ-linolenate produced dropped by 73% and 57%, respectively, from the control values, while the amount of oleate more than doubled.

Effects of Sulfoxide-Containing Fatty Acids. It is common for thioethers to undergo oxidation to sulfoxides in a variety of biological systems,[10] and we wondered if the sulfoxides corresponding to the thioether-containing fatty acids would have any inhibitory effects in cultures of *C. fasciculata*. Sulfoxides 6 and 7 were tested, but they are only very slight inhibitors of growth, and no significant effect on dihydrosterculate synthesis is observed.

Effects of Long-Chain Amides of 3-Amino-1,2-propanediol. In none of the experiments with the various free fatty acids were significant amounts of the inhibitors themselves observed in the total fatty acid mixtures, even when relatively high concentrations of the compounds were added to the growth medium. A derivative of the thioether-containing fatty acids was sought that would not be a substrate for β-oxidation (a possible mode of degradation of the free acids) but that would still be taken up by the cells and perhaps recognized by the enzymes to be inhibited. The long-chain amides 9 and 10, which resemble glycerol esters of the thioether-containing fatty acids, were synthesized for this purpose, and the stearic acid derivative 8 was prepared to control for the effect of the aminopropanediol moiety in these compounds.

Compound 8 has no effect on the growth of *C. fasciculata*. However, it does enter the cells, for as the concentration of 8 is increased in the growth medium, there is a parallel increase in the stearic acid fraction of the total fatty acids in the protozoa. No change in the distribution of the other cellular fatty acids is observed in the presence of 8. Compounds 9 and 10, derivatives of the 9- and 10-thiastearates, respectively, have effects similar to the thioether-containing free fatty acids in cultures of *C. fasciculata* (Table I). Compound 9 is a poor growth inhibitor, but it inhibits dihydrosterculate synthesis by 50% at about 8 μM. Compound 10 inhibits both growth and the synthesis of 1 at concentrations near 10 μM. The effects of these compounds on the distribution of the total fatty acids in the protozoa parallel those of the corresponding free acids.

Experimental Section

General Methods. Melting points, UV-visible absorption spectra, ^{1}H NMR spectra, and low- and high-resolution mass spectra were obtained as previously described.[11]

Growth of *C. fasciculata* and Inhibition Studies. Flasks containing 50 mL of the growth medium[5,12] and the desired concentration of fatty acid analogues or other additives were inoculated with aliquots of *C. fasciculata* (ca. 1×10^7 organisms). In typical experiments, duplicate flasks of medium were used for each of three to six concentrations of the analogue under study (including duplicate controls, with no additive). The cultures were incubated with shaking at 25 °C, and growth was monitored by changes in absorbance at 535 nm (relative to the uninoculated medium). An absorbance change of 1.0 corresponded to a cell density of 7×10^7 organisms/mL. Experiments were terminated when the control cultures achieved a cell density of 6×10^7 to 9×10^7 organisms/mL, and the cultures were then examined under a microscope to ensure that no contamination with other organisms had occurred. At this point the cells were harvested, the crude free fatty acids were isolated, and the fatty acid methyl esters were prepared as described before.[5]

Gas Chromatographic Analyses. Gas chromatographic (GC) analyses of mixtures of fatty acid methyl esters (FAMEs) were carried out with capillary columns [BP1 stationary phase, 25 m × 0.53 mm (Scientific Glass Engineering); Supelcowax 10 stationary phase, 15 m × 0.53 mm (Supelco)] on a Hewlett-Packard 5890A chromatograph with a flame-ionization detector. Both the BP1 and Supelcowax 10 columns were used for the analysis of all samples. Most C-12–C-24 FAMEs were resolved on the BP1 column by using a linear temperature program (180–270 °C, 2.5 °C/min). However, this system does not give a clean separation of the various C-18 FAMEs. This was accomplished by using the Supelcowax 10 column at 160 °C. The identities of the various components were established by co-injection with authentic standards and by coupled gas chromatography–mass spectrometry (GC–MS) performed on a Kratos MS50 instrument (BP1 capillary, 12.5 m × 0.32 mm; 180–270 °C, 2.5 °C/min). The purities of the fatty acid analogues used in this work were estimated by conversion of the compounds to their trimethylsilyl (TMS) ether/ester derivatives[11] followed by GC analysis with the BP1 capillary column.

Fatty Acids and Analogues. Stearic acid, oleic acid, linoleic acid, α-linolenic acid, and γ-linolenic acid were purchased from Sigma. 9,10-Methyleneoctadecanoic acid (1),[13] 9-thiastearic acid (3), 10-thiastearic acid (4), 8-(nonylsulfinyl)octanoic acid (6), and 9-(octylsulfinyl)nonanoic acid (7)[6] were synthesized as described previously. The purities of the sulfur-containing analogues above were estimated by GC analysis of the TMS esters to be as follows: 3, 99%; 4, 98%; 6, 98%; 7, 98%. The preparation of other analogues is described below, and the structures of all of the analogues are illustrated in Chart I.

8-Thiastearic Acid (2). A solution of 7-bromoheptanoic acid (2.00 g, 9.6 mmol) (Pfaltz & Bauer), 1-decanethiol (1.67 g, 9.6 mmol) (Aldrich), and KOH (1.07 g, 19.1 mmol) in ethanol (50 mL) was refluxed for 2 h under an argon atmosphere. After cooling, the solution was acidified, and the solvent was removed under reduced pressure. Ether and dilute HCl were shaken with the residue, and the organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated. Recrystallization of the residue from methanol gave 8-thiastearic acid (1.38 g, 4.6 mmol, 48%): mp 51–52 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 7 Hz, CH_3), 1.2–1.7 (m, 24 H, methylene envelope), 2.35 (t, 2 H, J = 7 Hz, CH_2COO), 2.50 (t, 4 H, J = 7 Hz, CH_2SCH_2); MS, m/z 302 (M^+, 20), 173 ($CH_3(CH_2)_9S^+$, 100), 143 ($M - H_2O - C_{10}H_{21}$, 32), 83 (37), 69 (41); exact mass 302.2265, calcd for $C_{17}H_{34}O_2S$ 302.2279. GC analysis of the TMS ester gave a purity of 96%.

11-Thiastearic Acid (5). Condensation of 10-bromodecanoic acid (Alfa) and 1-heptanethiol (Aldrich) in the above manner gave 11-thiastearic acid: mp 45–46 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 7 Hz, CH_3), 1.2–1.7 (m, 24 H, methylene envelope), 2.35 (t, 2 H, J = 7 Hz, CH_2COO), 2.50 (t, 4 H, J = 7 Hz, CH_2SCH_2); MS, m/z 302 (M^+, 31), 185 ($M - H_2O - C_7H_{15}$, 64), 131 ($CH_3(CH_2)_6S^+$, 100), 69 (59); exact mass 302.2283, calcd for $C_{17}H_{34}O_2S$ 302.2279. GC analysis of the TMS ester gave a purity of 97%.

***N*-(2,3-Dihydroxypropyl)octadecanamide (8).** Stearic acid (285 mg, 1.0 mmol) and (±)-3-amino-1,2-propanediol (100 mg, 1.1 mmol) (Aldrich) were placed in a Pyrex screw-capped tube under an argon atmosphere. The mixture was heated to 190 °C (oil bath) and maintained at that temperature for 1 h. After cooling, the reaction mixture was extracted with chloroform. This organic

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(11) Pascal, R. A., Jr.; Oliver, M. A.; Chen, Y.-C. J. *Biochemistry* **1985**, *24*, 3158–3165.
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(13) Grossert, J. S.; Ratnayake, W. M. N.; Swee, T. *Can. J. Chem.* **1981**, *59*, 2617–2620.

extract was washed with water, dried over anhydrous $MgSO_4$, and concentrated to dryness. The residue was redissolved in chloroform and stirred over solid sodium carbonate for 15 min. The mixture was filtered, and concentration of the resulting solution gave compound **8** as a white, crystalline solid (234 mg, 66%): mp 105-106 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz, CH_3), 1.2-1.8 (m, 30 H, methylene envelope), 2.23 (t, 2 H, J = 7 Hz, CH_2CO), 3.43 (m, 2 H, $CONHCH_2$), 3.58 (m, 2 H, CH_2OH), 3.78 (m, 1 H, $CHOH$), 5.93 (br, 1 H, NH); MS, m/z 357 (M^+, 10%), 326 (M - CH_2OH, 55), 297 (M - CH_2OH - CHO, 21), 284 ($CH_3(CH_2)_{16}CONH_3^+$, 28), 267 ($CH_3(CH_2)_{16}CO^+$, 22), 133 (100), 115 (61); exact mass 357.3249, calcd for $C_{21}H_{43}NO_3$ 357.3243. GC analysis of the TMS ether derivative indicated a purity of 94%.

***N*-(2,3-Dihydroxypropyl)-8-(nonylthio)octanamide (9).** 9-Thiastearic acid and (±)-3-amino-1,2-propanediol were condensed as described above to give compound **9**: mp 91-93 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz, CH_3), 1.2-1.8 (m, 24 H, methylene envelope), 2.25 (t, 2 H, J = 7 Hz, CH_2CO), 2.53 (t, 4 H, J = 7 Hz, CH_2SCH_2), 3.45 (m, 2 H, $CONHCH_2$), 3.56 (m, 2 H, CH_2OH), 3.78 m, 1 H, $CHOH$), 5.94 (br, 1 H, NH); MS, m/z 376 (M + H, 2%), 357 (M - H_2O, 13), 344 (M - CH_2OH, 12), 285 ($CH_3(CH_2)_8S(CH_2)_7CO^+$, 35), 248 (45), 216 (100), 133 (73), 92 (68); exact mass (M - H_2O) 357.2705, calcd for $C_{20}H_{39}NO_2S$ 357.2701. GC analysis of the TMS ether derivative indicated a purity of 85%; the principal contaminant was the acid **3**.

***N*-(2,3-Dihydroxypropyl)-9-(octylthio)nonanamide (10).** 10-Thiastearic acid and (±)-3-amino-1,2-propanediol were condensed as described above to give compound **10**: mp 89-91 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz, CH_3), 1.1-1.8 (m, 24 H, methylene envelope), 2.24 (t, 2 H, J = 7 Hz, CH_2CO), 2.53 (t, 4 H, J = 7 Hz, CH_2SCH_2) 3.43 (m, 2 H, $CONHCH_2$), 3.56 (m, 2 H, CH_2OH), 3.78 (m, 1 H, $CHOH$), 5.99 (br, 1 H, NH); MS, m/z 376 (M + H, 3%), 357 (M - H_2O, 32), 344 (M - CH_2OH, 20), 285 ($CH_3(CH_2)_8S(CH_2)_7CO^+$, 75), 262 (71), 230 (50), 146 (68), 133 (94), 92 (100); exact mass (M - H_2O) 357.2708, calcd for $C_{20}H_{39}NO_2S$ 357.2701. GC analysis of the TMS ether derivative indicated a purity of 94%.

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Registry No. **2**, 114692-26-1; **3**, 106689-24-1; **4**, 105099-89-6; **5**, 114692-27-2; **6**, 106689-25-2; **7**, 106689-26-3; **8**, 7336-25-6; **9**, 114692-28-3; **10**, 114692-29-4; stearic acid, 57-11-4; linoleic acid, 60-33-3; γ-linolenic acid, 506-26-3; dihydrosterculic acid, 5711-28-4; oleic acid, 112-80-1.

2-(2-Aryl-2-oxoethylidene)-1,2,3,4-tetrahydropyridines. Novel Isomers of 1,4-Dihydropyridine Calcium Channel Blockers[1]

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The title compounds are novel double bond isomers of 1,4-dihydropyridine-type calcium channel blockers (CCB). These derivatives were prepared by using the Hantzsch dihydropyridine synthesis. The assignment of structure was based on spectroscopic data and a regiochemically unambiguous synthesis. Several of the analogues inhibited [^{3}H]nitrendipine binding with IC_{50} values as low as 25 nM. By comparison, nifedipine, a clinically useful 1,4-dihydropyridine CCB, inhibits [^{3}H]nitrendipine binding with an IC_{50} of 1.6 nM. In the Langendorff rat heart preparation, treatment with the more potent derivatives produced marked dose-related increases in coronary flow with little or no effect on heart rate or contractility, except at the highest concentrations tested. The selectivity for vascular versus cardiac effects was similar to that of nifedipine, i.e. the concentration producing vasodilation was approximately 2 orders of magnitude lower than the concentration eliciting cardiodepression. These novel isomers extend the structure–activity relationships for calcium channel blockers into a series closely related to the 1,4-dihydropyridines.

Verapamil, diltiazem, and nifedipine are the best known examples of calcium channel blockers, compounds that inhibit cellular calcium influx through the voltage-dependent calcium channel. Each of these three compounds represents a distinctly different chemical class and interacts with a discrete binding site linked to the calcium channel.[2,3] Nifedipine, **1**, is the prototype for the 1,4-dihydropyridine class of calcium channel blockers. A large number of 1,4-dihydropyridine analogues have been reported allowing delineation of well-defined structure–activity relationships (SAR). Among the requirements for potent receptor binding and calcium channel blockade are (a) N-1 hydrogen, (b) an ester at C-3 or C-5, preferably both, (c) an aryl group at C-4, optimally substituted with an ortho or meta electron-withdrawing group, (d) small alkyl groups at C-2 and C-6, and (e) a dihydropyridine ring (rather than pyridine or tetrahydropyridine).[4-8] Studies involving X-ray crystallographic analysis[9] and rigid analogues[10] suggest that the conformation of the 1,4-dihydropyridine ring is a shallow boat with the 4-aryl group oriented perpendicular to and bisecting the plane of the dihydropyridine ring.

Recently, relatively small structural modifications have been shown to produce 1,4-dihydropyridine derivatives that activate calcium channels. Bay k 8644, **2**, is the first

[†] Department of Chemistry.
[‡] Department of Pharmacology.

(1) This work was presented in part at the 195th National Meeting of the American Chemical Society, New Orleans, LA, August 30-September 4, 1987. See: *Abstracts of Papers*; American Chemical Society: Washington, DC, 1987; MEDI 83.
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