loss of a $C_1$ unit from the side chain, and compound (**13**), deriving from a nuclear dehydroxylation reaction, appear in similar amounts up to 19 days.

Synapic acid (**11**) (scheme 3) was transformed after a 9-day incubation into 3,5-dimethoxy-4-hydroxyphenylpropionic acid (**16**); 3,5-dimethoxyphenylpropionic acid (**17**); 3,5-dimethoxyphenylacetic acid (**18**) and 3,5-dimethoxy-4-hydroxyphenylacetic acid (**19**). After a 24-day incubation compounds (**16**), (**17**) and (**18**) were detected.

A similar anaerobic transformation was shown to occur also incubating 4-hydroxy phenylpropionic acid (**20**) and p-coumaric acid (**2**) for 12 days with the consortium.

From these experimental data it seems that three reactions are involved in the anaerobic catabolism of the tested hydroxycinnamic acids:

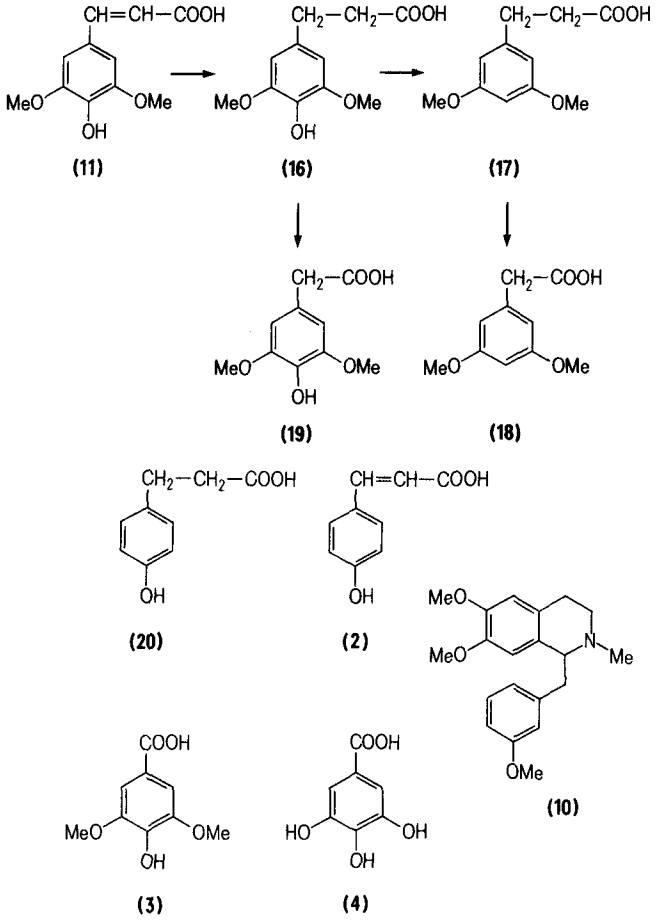
1) The reduction of the double bond of the side chain;
2) the replacement of the nuclear hydroxyl group in position 4 by hydrogen;
3) the demolition of the side chain by the loss of a $C_1$ unit.

Ethylbenzenes and styrenes deriving from the direct decarboxylation of the side chain were never observed.

The data shown in figure 1 for caffeic acid suggest that compound (**7**), formed by a $C_1$ unit loss from the side chain, is rapidly dehydroxylated to compound (**9**), and that compound (**8**), formed by a dehydroxylation reaction, does not undergo further degradation by a $C_1$ loss from the side chain at a comparable rate. Since compounds (**7**) and (**8**) derive from the common intermediate (**6**), it can be concluded that the nuclear dehydroxylation reaction of caffeic acid, carried on by our adapted consortium, is more rapid than the $C_1$ loss from the side chain.

The presence of the meta hydroxylated compounds (**8**) and (**9**) suggests that the dehydroxylation reaction occurs mainly at the para position.

Scheme 3

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## 5-(Hydroxyimino)-4-methoxy-2-(pivaloylimino)thiazolidine-3-acetamide, a reduced nitroheterocyclic derivative with potent schistosomicidal properties

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*23 August 1984*

*Summary.* The synthesis and antischistosome properties of 5-(hydroxyimino)-4-methoxy-2-(pivaloylimino)thiazolidine-3-acetamide (**1**) are described. The compound was prepared by reduction of the nitrothiazoline (**2**) with stannous chloride in methanol, and represents the first example of a reduced nitroheterocyclic compound showing potent schistosomicidal properties. The possible relationship of compounds such as **1** to the as yet unidentified reduced active but toxic entities formed in vivo from nitroheterocyclics like metronidazole is discussed.

*Key words.* Metronidazole; methoxy-oximinothiazolidine; reduced nitroheterocyclic; schistosomicidal; *Schistosoma mansoni.*

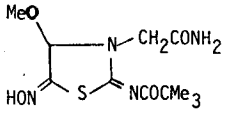
A range of nitroheterocyclic and nitroaromatic compounds have been shown to possess antischistosome properties[2], and indeed several clinically used drugs of this type have emerged with good efficacy against human *Schistosoma* infections[2b]. In addition, these and many other nitro compounds are variously active against a wide variety of helminths, protozoa, fungi and bacteria.

Although many investigations have been made into the mode of action of antiparasitic nitroheterocyclics, the precise identity of the active entity has yet to be established. Nevertheless, to date, all derivatives of the active nitro compounds in which the nitro group has been reduced appear to be inactive in the screens where the parent nitro compound is active.

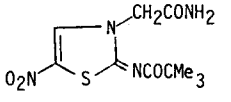
Extensive earlier investigations have shown that certain 5-nitro-2-thiazolines possess extremely potent schistosomicidal properties in both rodents and primates[3]. We wish to report here that

the oxime **1**, prepared by stannous chloride reduction of 2-(pivaloylimino)-5-nitro-4-thiazoline-3-acetamide **2**, is in fact a potent schistosomicidal agent in mice infected with *Schistosoma mansoni*.

*Chemistry.* Slow addition of anhydrous stannous chloride (0.5 moles) to a solution of the nitrothiazoline **2**[3a] (0.1 moles) and anhydrous sodium acetate (1 mole) in methanol at room temperature gave, after removal of inorganic salts, evaporation of solvent, and trituration with ethyl acetate, crude 5-(hydroxyimino)-4-methoxy-2-(pivaloylimino)thiazolidine-3-acetamide **1** (19%). Recrystallization (charcoal) from methanol afforded (poor recovery) pure **1**, m.p. 180–182 °C (found: C, 43.7; H, 6.1; N, 18.4. $C_{11}H_{18}N_4O_4S$ requires C, 43.7; H, 6.0; N, 18.5%); $\delta$(DMSO-$d_6$), 1.11 (s, 9H, t-Bu), 3.25 (s, 3H, OMe), 3.97 and 4.36 (d, 2H, $J$ 16.3 Hz, $NCH_2$), 5.66 (s, 1H, CH), 7.20 (s, 1H, amide NH), 7.57 (s, 1H, amide NH), and 12.25 (s, 1H, =NOH).

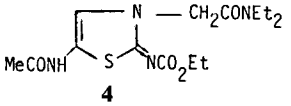



**1**

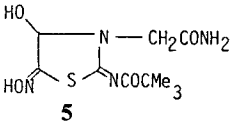



**2**

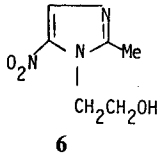
$Me_3CCONHCSNHCH_2CONH_2$ **3**



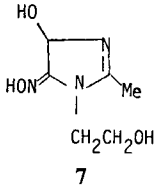

**4**




**5**




**6**




**7**

A by-product also isolated in low yield from the reaction mixture was shown to be the open-chain thiourea **3** (m.p. 157–159 °C from ethanol).

*Biology.* The oxime **1** was tested against 9-week mature infections of *S. mansoni* in Keeble or Charles River CD1 white mice by p.o. or s.c. administration and the results are presented in the table.

*Discussion.* Clearly, the table shows that the oxime **1** has good potency against *S. mansoni* in mice on both oral and parenteral administration. Indeed, the compound appears to be at least as potent as niridazole when given orally. This is in contrast with the only other reduced nitrothiazoline-3-acetamide (**4**) prepared to date[3a], which was inactive against *S. mansoni* although the parent nitro compound was active[3a].

Efficacy of **1** against *S. mansoni* in mice

| Drug | Route × days | Dose (mg/kg/day) | %kill of *Schistosomes* |
|---|---|---|---|
| **1** | SC × 5 | 50 | 95 |
| | PO × 5 | 50 | 78 |
| | SC × 1 | 50 | 62 |
| | PO × 1 | 50 | 16 |
| Niridazole | PO × 1 | 50 | 0 |
| | PO × 5 | 50 | 64 |
| Praziquantel | PO × 1 | 50 | 13 |

As might be expected, the by-product thiourea **3** was not active in the mouse primary screen. It is postulated that this compound is formed from traces of water present in the reaction mixture through the intermediacy of the (probably highly reactive) hydroxy-oxime **5** (which could not be isolated). In confirmation of this, when the stannous chloride reduction of **2** was effected in aqueous tetrahydrofuran instead of methanol, greatly increased yields of **3** (and no **1**) were obtained.

When thiazoline **2** was administered to rats at single oral doses of 600 mg/kg, thiourea **3** was isolated from plasma as a major metabolite[4]. Again this compound could have been formed from **2** through the putative carbinolamine intermediate **5**.

Finally, although it is not known whether species such as **5** possess schistosomicidal properties, clearly the 'trapped' methyl ether **1** is active. It is tempting, however, to speculate on the relationship between the suggested novel intermediate **5**, and the nature of the biologically active (and cytotoxic), as yet unidentified, in vivo primary reduction products of nitroimidazoles such as metronidazole **6** and other antiparasitic nitroheterocyclics. Certainly, the known ring-opened metabolites of metronidazole [acetamide and N-(2-hydroxyethyl)oxamic acid[5,6]] could result from hydrolytic cleavage of a putative imidazoline-intermediate (**7**) corresponding to **5**. Further experiments are in hand to investigate the reduction of metronidazole using the conditions described for the synthesis of **1**.

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## The repression of siderophore synthesis by Mt. St. Helens' ash and silicate rock standards

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*Summary.* Mt. St. Helens' ash or U.S. Geological Survey silicate rock standards would replace $FeCl_3$ as the limiting nutrient and repress the synthesis of rhodotorulic acid in *Rhodotorulia pilimanae* cultures.

*Key words.* Siderophore; *Rhodotorula pilimanae;* rhodotorulic acid; Mt. St. Helens' ash.

To overcome the limited solubility of iron salts many microbes derepress the synthesis of ferric specific chelators (siderophores). These chelators are often excreted into the environment where following iron acquisition the complex is absorbed. Substantial information is known on siderophore biochemistry[2], but little is known on the sources or iron available to siderophores. The