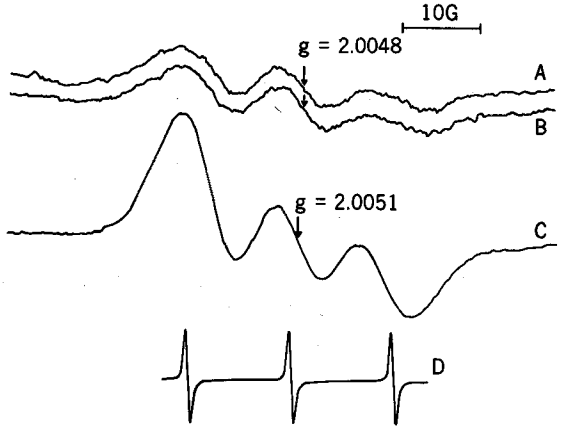
phosphate buffer. Electron Spin Resonance (ESR) spectra were obtained at room temperature using a Varian E-9 X-band system operating at 100 kHz.

Our novel results are presented in the figure. NOF reacted with squalene, oleic acid and linoleic acid to produce free radicals as indicated by the ESR spectra. The 3-line-spectrum in each case and the g values suggest that the free radicals produced are nitroxyl free radicals (also see below) with nitrogen splitting constants of 11.85 gauss for both linoleic and oleic acid and 11.35 gauss for squalene. All hydrocarbons containing carbon-carbon double bonds that we have tested thusfar including purified egg lecithin suspended as liposome vesicles react with NOF to yield ESR spectra very similar to those shown in the figure. However, all hydrocarbons lacking a carbon-carbon double bond that we have tested do not yield a free radical spectrum. We have found that the amount of free radical formed tends to follow the number of double bonds in the lipid molecule. This is not a strict relationship, however, and we believe the positioning of the double bonds in relation to each other is also very important. A more detailed treatment of our observations will be published; but for the present communication, it is important to point out that EDTA (ethylenediamine tetraacetic acid) did not influence the amount of free radicals formed or the reaction rate. Therefore we believe the reaction occurs directly without a catalyst (such as a trace of metal ions) being necessary.



ESR spectra obtained after 2-nitrosofluorene (NOF) was allowed to react with various unsaturated lipids. NOF (0.25 μmoles) was added to 2 ml of potassium phosphate buffer containing: a) 3.57 μmoles of linoleic acid, b) 3.57 μmoles of oleic acid and c) 2.44 μmoles of squalene. The ESR sweep was started 20 sec after the reaction was initiated by addition of the lipid in a small amount of methanol. Under these conditions, the amount of free radical reached a maximum after about 15 min and stayed relatively constant in amount for the next hour. The ESR conditions were: sweep time, 100 gauss in 30 min with a filter constant of 10 sec, frequency 9.5305 GHz at 15 mW incident power, modulation 8 gauss at 100 kHz, temperature 25 °C. Spectrum D is that of the Fremy's salt standard. No free radical signal was obtained with either NOF only in buffer or with any one of the lipids alone in buffer.

Our results were very surprising but a thorough search of the literature revealed that Sullivan[10] had observed a somewhat similar reaction in 1966 in that nitrosobenzene reacted directly with 2,3-dimethylbutene to yield stable nitroxyl free radicals having g values and nitrogen splitting constants similar to the ones we report here. He postulated that the reaction occurred via a 'novel pseudo Dield-Alder' mechanism in which the hydroxylamine was an intermediate and that oxygen and/or nitrosobenzene oxidized the hydroxylamine to the nitroxyl free radical form. Knight succeeded in isolating the hydroxylamine intermediate in good yield 4 years later[11]. We have found that oxygen is not required in the reaction described here and that under these conditions N-hydroxy-2-aminofluorene (i.e. reduced NOF) is formed in the reaction. Therefore, because of these and many other observations to be described in detail later we believe NOF adds directly to the carbon-carbon double bond of a hydrocarbon producing the hydroxylamine intermediate which is then oxidized to the nitroxyl free radical.

It is clear from the results presented here that NOF when formed in vivo will react with lipids containing double bonds to produce a novel form of the carcinogen. The true significance of this reaction to the understanding of AAF carcinogenesis must await further investigation. It should, however, be noted that Stier et al.[12] observed nitroxyl free radicals having properties somewhat similar to the free radical observed here in the chloroform-methanol extract of rabbit liver microsomes metabolizing AAF. All of these observations tend to indicate that the reaction reported here does occur in vivo and point to a need to understand the nature of this reaction in greater detail as well as its significance to AAF carcinogenesis.

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Nitrothiophenes with schistosomicidal activity

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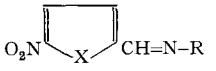
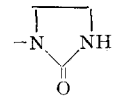
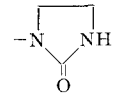
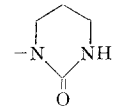
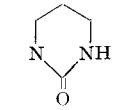
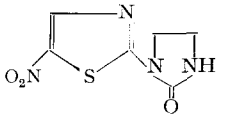
Summary. A series of nitrothiophene compounds, with activity against Schistosoma mansoni in mice, is described and shown to be more effective than the corresponding nitrofurans.

Investigations carried out to determine the structural requirements of nitroheterocyclic antischistosome compounds have so far indicated that activity can be found in nitroimidazoles[1], nitrothiazoles[2], nitrofurans[3] and nitrothiophenes[4]. Of these, the nitroimidazoles have produced oogram changes only; within the nitrothiazoles and nitrofurans, the range of active compounds has been limited to certain specific structural features[3–5] and the only nitrothiophenes to exhibit even weak activity have been the analogues of active nitrofurans[4].

The majority of nitroheterocyclic compounds tested do not possess any significant antischistosome action, and this lack of effect has been attributed by Bueding and his co-workers[3–5] to the absence of such structural necessities

Table 1. The schistosomicidal activity of nitrofurans and nitrothiophenes of general structure: 

Compound structure X	R	Antischistosome activity Total adult S. mansoni	Number of dead worms	% kill
O		161	0	0
S		152	18	12
O		149	0	0
S		129	109	84
	 (Niridazole)	71	41	58
Control mice		112	0	0

There were 7 mice in each group, and the figures given refer to the total number of schistosomes found per group. All compounds were dosed once per day on 4 consecutive days – 250 mg/kg orally on the first 2 days, and 25 mg/kg intraperitoneally on the third and fourth day.

as a nitrogen-containing substituent attached directly, or via a rigid side chain, to the 2-position of the nitroheterocyclic ring.

However, the conformational limitations laid down by Bueding et al. did not preclude the possibility of retaining activity on the exchange of nitrothiophene for nitrofuran in any group of compounds, although Henry et al.[4] gave three examples where such an exchange was disadvantageous in terms of antischistosomal action.

On investigating this substitution of Sulphur and Oxygen in a different type of nitrofuran, in which the bridging group was methyleneimine rather than vinyl or carboxamide, it was found that the antischistosome activity altered from that which only affected the production of viable eggs[6], to that which killed adult Schistosoma mansoni harboured in the venous portal complex of mice (table 1).

This increased lethality was particularly apparent for the change from 1-(5-nitro-2-furfurylideneamino)-tetrahydro-2(1H)-pyrimidone to its nitrothenylidene amino analogue, which enhanced the kill of schistosomes from zero to a level comparable to that achieved with niridazole, the activity of which is well proven.

A structure-activity relationship for these nitrothenylideneamino compounds was first sought by varying the

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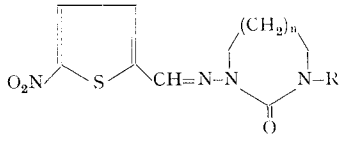
Table 2. The effect of 3′-position substitution on the schistosomicidal activity of nitrothenylideneamino-tetrahydro pyrimidones and -imidazolidinones

General structure: 

3′-substituent R	Tetrahydro pyrimidones (n = 1) Total S. mansoni	Dead worms	% kill	Imidazolidinones (n = 0) Total S. mansoni	Dead worms	% kill
H	97	97	100	213	33	15
CH_3	101 (135)	44 (49)	44 (36)	99	47	47
C_2H_5	146	131	90	94	90	96
n-C_3H_7	93	57	61	209	8*	4
iso-C_3H_7	118	38	32	79	0**	0
n-C_4H_9	164 (133)	114 (33)	69 (25)	–	–	–
n-C_5H_{11}	147	17	12	–	–	–
CH_3CO–	126	0	0	225 (87)	147 (80)	65 (92)
C_2H_5CO–	–	–	–	164	2	1
$HOCH_2CH_2$–	–	–	–	222	9	4
$C_6H_5CH_2$–	–	–	–	145	0	0
C_6H_5CO–	152	0	0	–	–	–
CH_3OCH_2–	159	109	69	–	–	–
Niridazole	66	66	100			
Control group	137	0	0			

All compounds were dosed orally at 250 mg/kg once daily for 4 consecutive days except for * where 200 mg/kg twice daily for 4 days was given and ** where only 3 × 250 mg/kg was administered because of the toxicity to the host mice. Figures in parentheses are the results of duplicate experiments.

substituent at the 3'-position of the pyrimidone or imidazolidinone ring. The effect of such substitution on schistosomicidal action is shown in table 2. In the pyrimidone series, the unsubstituted compound, the 3-methoxymethyl, the 3-ethyl and the 3-n-propyl derivatives were the most active, whereas the 3-ethyl and 3-acetyl were the best of the imidazolidinone series. The inactivity of the 3-acetylpyrimidone and the 3-n-propylimidazolidinone made any correlation for the 2 series difficult to conceive.

In all tests, the lethality of the compound was judged by the number of dead or dying schistosomes encapsulated within the substance of the host liver, compared to the total number of worms found in the liver and portal system after perfusion and examination of a liver squash preparation.

The most active compounds have been given to infected mice as single oral doses ranging from 300 to 1000 mg/kg. The percentage kill of schistosomes was linear with respect to $\log_{10}$ dose, and the following ED_{50} results were obtained (compound I): 1-(5-nitro-2-thenylideneamino)-tetrahydro-2(1H)-pyrimidone, 334 mg/kg with 95% confidence limits of 312 and 357: 1-(5-nitro-2-thenylideneamino)-3-ethyl-2-imidazolidinone, 450 mg/kg (95% limits 363 and 557); 1-(5-nitrothiazolyl)-2-imidazolidinone (niridazole), 443 mg/kg (95% limits 387 and 507). Thus, the nitrothiophenes compared favourably with niridazole under these conditions. In the course of these studies, it was found that none of the mice (8/group) exhibited any overt toxic symptoms after single oral doses of 1000 mg/kg. In a separate test groups of 8 mice also tolerated 200 mg/kg given twice daily for 4 consecutive days. All of these mice harboured S. mansoni in varying numbers and were generally in better condition than undosed mice when examined. However, LD_{50} values have not been established for either healthy or parasitized mice.

The most active pyrimidone (compound I) has also been tested against S. mansoni harboured in Syrian hamsters. A dose regime of 300 mg/kg daily for 4 consecutive days produced a kill of 29% (39 of a total of 134 worms recovered from 6 hamsters were dead), while a single dose of 750 mg/kg gave an average of 27% kill in another group of 6 hamsters. Preliminary investigations of the metabolism of compound I in mice and hamsters indicated that biotransformation and excretion was more rapid and extensive in the hamsters, which may explain the lowered effectiveness in that species.

These findings, a) that certain nitrothiophene compounds possess schistosomicidal activity, and b) that a methyleneimine bridging group gives a correct configuration for activity, both extend and modify the concept of Robinson, Bueding and Fisher[3] as to the structural limitations imposed upon nitroheterocyclic compounds if they are to have antischistosomal action.

Development of photochemical activity during greening of heat-stressed etiolated seedlings of Zea mays

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Summary. When etiolated maize seedlings were subjected to a heat stress of 40 or 45 °C for 16 h, and subsequently illuminated, photoreduction of ferricyanide and noncyclic photophosphorylation by chloroplasts isolated therefrom were retarded, and the lag in the appearance of these photochemical reactions was extended.

Introduction. The accumulation of photosynthetic pigments and the development of photosynthetic activity in chloroplasts isolated from greening etiolated plants have been investigated[1–7]. In previous communications, we reported that the rates of formation of chlorophylls[8,9], and protochlorophyll[10], and the rates of cyclic photophosphorylation with phenazine methosulfate and of ATP hydrolysis[11] of such chloroplasts were retarded if the etiolated plants had been subjected to high temperatures before greening. In this report, the development of 2 other photochemical activities of chloroplasts i.e. reduction of ferricyanide and noncyclic photophosphorylation with ferricyanide during greening of heat stressed dark-grown maize seedlings is described.

Materials and methods. Seeds of maize (Zea mays Linn. cv NS1) were germinated at 25 °C in darkness on sand. The resulting seedlings were daily supplied with distilled water. On the 4th day after sowing, different lots of seedlings were subjected to temperatures of 40 or 45 °C for 16 h in darkness. The seedlings were then returned to 25 °C and illuminated at 3000 lux intensity supplied by small fluorescent tubes.

At intervals of 4, 6 or 8 h after the onset of illumination, chloroplasts were extracted by the method of Howes and Stern[12] from leaves harvested randomly from each treatment. Light reactions were carried out in a glass-sided water bath which was illuminated on each side by 2 300 W reflector spot lamps. Temperature was maintained at 24 °C by means of an electric cooling coil immersed in the water bath. Reactions proceeded for 2 min in the light (80,000 lux) or dark and were terminated by turning the light off, where appropriate, and adding trichloroacetic acid to a final concentration of 3% (w/v). After centrifugation, aliquots of the supernatants were assayed for ferricyanide reduction and noncyclic photophosphorylation.

The standard reaction mixture contained in μmoles: tris (pH 8.0), 135; $MgCl_2$, 24; ADP, 12; NaK phosphate (pH 8.0), 36; BSA, 0.135; $K_3Fe(CN)_6$, 4.5; chloroplasts containing 75–150 μg chlorophyll; and water to a final volume of 9 ml.

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