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Antiparasitic Nitroimidazoles. 3. Synthesis of 2-(4-Carboxystyryl)-5-nitro-1-vinylimidazole and Related Compounds

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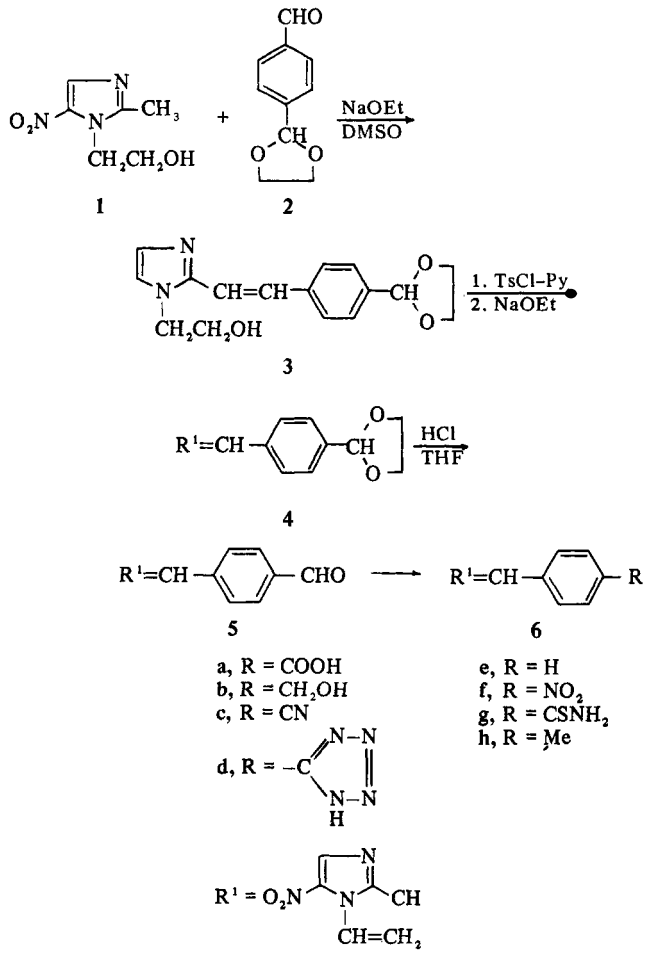
The synthesis of 6 (R = COOH), one of its metabolities (R = $CONHCH_2COOH$), and 31 related compounds is described. The compounds were examined for antiparasitic activity against *Trichomonas vaginalis* and *Entamoeba histolytica in vitro* and *in vivo* and against various *Trypanosoma* species *in vivo*. The compounds were also tested against *Schistosoma mansoni* in mice and hamsters. Comparisons are made with standard drugs.

The need for new classes of drugs effective against the African trypanosomiases has been stressed in specialist publications during the last few years.[1,2] In part I[3] of this series of papers, we described the antiprotozoal activity of a series of 2-styryl-5-nitroimidazoles emphasizing in particular their antitrypanosomal properties. A related paper† discusses the metabolism, in various species, of several of these styrylimidiazoles and describes the isolation and identification of a metabolite, 2-(4-carboxystyryl)-5-nitro-1-vinylimidazole (**6a**). This compound, its β-glucuronide, and its glycine conjugate were isolated from the urine of mice, rats, rabbits, hamsters, and dogs† after oral or parenteral dosing of 2-(4-methylstyryl)-5-nitro-1-vinylimidazole[3] (**6h**). In this paper we describe the synthesis and antiparastic activity of **6a** and various related compounds. As we were uncertain as to whether the acid **6a** or the alcohol **6b** were active metabolites (*cf.* lucanthone-hycanthone),[4] a synthesis was devised which was capable of yielding either compound (Scheme I).

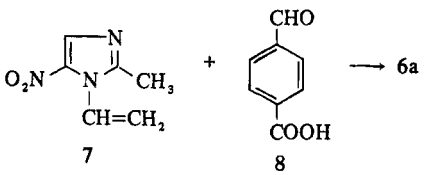
Scheme I



Although **2** was readily prepared, purification by distillation under reduced pressure was not possible due to concomitant disproportion into terephthaldehyde and its bisethylene acetal. However, the base-catalyzed condensation of **2** with metronidazole **1** gave the styrylimidazole **3** which was converted to the *N*-vinyl compound as shown in Scheme I.

Acetal **4** underwent acid-catalyzed cleavage to the aldehyde **5** which on oxidation[5] gave a high yield of the acid **6a** while reduction with $NaBH_4$ gave the alcohol **6b**.

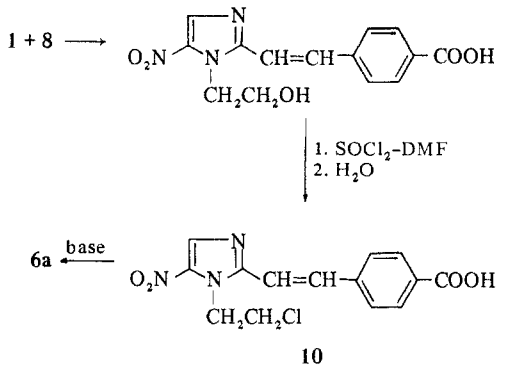
Compound **6a** could also be prepared by direct condensation of **7**[3] with 4-carboxybenzaldehyde (**8**) in the presence of base, but the reaction was capricious due to the instability of **6a** under the strongly basic conditions.[6]



Condensation of **1** with **8** (Scheme II) gave **9** which was readily converted to the chloride **10** on treatment with the DMF-$SOCl_2$ complex[7] followed by hydrolysis. Dehydrohalogenation of **10** with a variety of bases gave **6a** in poor yield.

†D. M. Morton and J. N. Green, unpublished results.

Scheme II

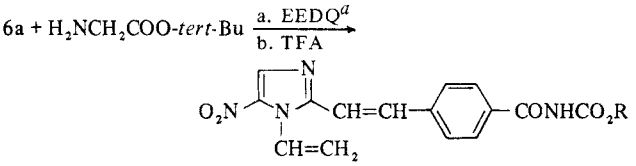


10

The nitrile **6c** was prepared from the oxime of aldehyde **5** and converted to the tetrazole **6d** by a literature method.[8] The thioamide **6g** was prepared from **6c** using the method described by Taylor and Zoltewicz.[9] Nitration of **6e**[3] gave **6f** as shown by the nmr spectrum which was consistent with a para-disubstituted benzene.

The esters and amides listed in Table I were prepared by treatment of the acid chloride of **6a** with the appropriate alcohol or amine in a suitable solvent. The glycine conjugate **11b** was prepared as shown in Scheme III and was found to be identical (ir and mass spectrum) with a metabolite of **6a** isolated from the urine of rats, rabbits, etc., which had been dosed with **6a**.[†]

Scheme III

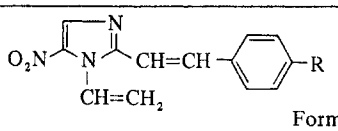


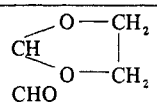
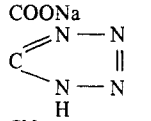
11 a, R = *tert*-Bu
b, R = H

[a] 1-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline.

Biological Results. Trichomoniasis. Table I shows that nearly all the compounds inhibit the growth of *Trichomonas vaginalis in vitro* at similar levels to metronidazole.

Table I



Compd	R	Yield, %	Crystn solvent	Mp, °C	Formula analysis[a]	MIC, μg/ml[b] T. vaginalis	MIC, μg/ml[b] E. histolytica
4		25	EtOAc	135-136	$C_{16}H_{15}N_3O_4$	1.0	32
5	CHO	99	EtOAc	196-197	$C_{14}H_{11}N_3O_3$	2.0	10
6b	CH_2OH	71	EtOAc	204-205	$C_{14}H_{13}N_3O_3$	1.0	10
6a	COOH	81	Dioxane	303-306 dec	$C_{14}H_{11}N_3O_4$	0.25	16
12	COONa	77	H_2O-*n*-BuOH		$C_{14}H_{10}N_3O_4Na$	<1	32
6d		14	H_2O-MeOH	203-204 dec	$C_{14}H_{17}N_7O_2$	2	100-1000
6c	CN	69	EtOAc	215-216 dec	$C_{14}H_{10}N_4O_2$	<1	1-10
6f	NO_2	52	Me_2CO	212-213	$C_{13}H_{10}N_4O_4$	<1	10-100
13	CO_2Me	69	EtOAc	207-208	$C_{15}H_{13}N_3O_4$	2.0	>1000
14	CO_2-*n*-Bu	72	Me_2CO	104-105	$C_{18}H_{19}N_3O_4$	1.0	100-1000
15	$CO_2(CH_2)_2NEt_2 \cdot HCl$	51	EtOH-HCl	216-217	$C_{20}H_{24}N_4O_4 \cdot HCl$	2	32
16	$CO_2CH(Me)CH_2NMe_2 \cdot HCl$	87	EtOH-HCl	245-246	$C_{19}H_{22}N_4O_4 \cdot HCl$	0.5	100-1000
17	$CO_2(CH_2)_2$-c-$N(CH_2CH_2)_2O$	64	EtOAc	160-161	$C_{20}H_{22}N_4O_5$	0.5	64
18	$CO_2(CH_2)_2$-c-$N(CH_2)_4$	62	EtOAc	120-121	$C_{20}H_{22}N_4O_4$	0.5	100-1000
19	$CO_2(CH_2)_2$-c-$N(CH_2)_5$	63	EtOAc	151-152	$C_{21}H_{24}N_4O_4$	0.5	100-1000
20	$CO_2(CH)_2$-c-$N(CH_2)_5 \cdot HCl$	68	EtOH-HCl	230-232 dec	$C_{21}H_{24}N_4O_4 \cdot HCl$	0.5	100-1000
21	$CONH_2$	85	DMF-H_2O	247-248	$C_{14}H_{12}N_4O_3$	0.25	32
6g	$CSNH_2$	90	DMF-H_2O	218-220 dec	$C_{14}H_{12}N_4O_2S$	4	64
22	CONHMe	73	EtOAc	199-200	$C_{15}H_{14}N_4O_3$	0.5	64
23	CONHEt	75	EtOAc	173-174	$C_{16}H_{16}N_4O_3$	0.25	16
24	$CONH(CH_2)_2OH$	75	EtOAc	182-183	$C_{16}H_{16}N_4O_4$	0.5	64
25	CONHCH(Me)(Et)	75	EtOAc-petroleum ether	167-168	$C_{18}H_{20}N_4O_3$	0.5	64
26	CONH-c-$(CH_2)_3$	72	EtOAc	180-181	$C_{17}H_{16}N_4O_3$	0.5	32
27	$CONHCHMe_2$	76	EtOAc	194-195	$C_{17}H_{18}N_4O_3$	0.25	64
28	$CONH(CH_2)_2$-c-$N(CH_2CH_2)_2O$	47	$CHCl_3$-petroleum ether	179-180	$C_{20}H_{23}N_5O_4$	2.0	100-1000
29	$CONH(CH_2)_2$-c-$N(CH_2)_5$	55	$CHCl_3$-petroleum ether	157-159	$C_{21}H_{25}N_5O_3 \cdot 0.5H_2O$	2.0	100-1000
30	$CON(Et)_2$	82	EtOAc	161-162	$C_{18}H_{20}N_4O_3$	0.25	32
31	$CON(CHMe)_2)_2$	59	EtOAc	251-252	$C_{20}H_{24}N_4O_3$	8.0	64
32	$CON(n\text{-}Bu)_2$	68	EtOAc	116-117	$C_{22}H_{28}N_4O_3$	2.0	100-1000
33	CO-c-$N(CH_2CH_2)_2O$	63	EtOAc	214-215	$C_{18}H_{18}N_4O_4$	0.5	100-1000
34	CO-c-$N(CH_2)_4$	61	EtOAc	161-162	$C_{18}H_{18}N_4O_3$	8.0	16
11a	$CONHCH_2CO_2$-*tert*-Bu	35	EtOAc	188-190	$C_{20}H_{24}N_4O_5$	4.0	64
11b	$CONHCH_2COOH$	88	DMF-H_2O	268-270 dec	$C_{16}H_{14}N_4O_5$	0.5	100-1000
1	Metronidazole					0.5	32

[a] All compounds analyzed for C, H, and N. [b] Obtained by serial dilution.

Table II

Compd	% activity,[a] *T. vaginalis* mice, 20 mg/kg × 5 po
4	58
5	72
6b	82
6a	100
12	100
15	54
23	60
6h	50
Metronidazole (**1**)	100

[a]Per cent activity is calculated from the extent of visible diminution of diffuse visceral lesions together with reduction of parasites present in lesions. In this test normal and infected controls were included.

The compounds which showed reasonable activity against *T. vaginalis* in mice at 20 mg/kg × 5 po when tested according to the method described by Honigberg[10] are listed in Table II. Compounds which resulted in less than a 50% reduction in lesion score, compared to the lesion score in untreated, infected mice, were considered inactive. The free carboxyl function of compound **6a** appears necessary for good activity against *T. vaginalis in vivo*, in keeping with the results obtained by Tarrant, Green, and coworkers.[11] These indicated that high initial levels of **6a** would be necessary to kill *T. vaginalis*. Presumably the hydrolysis of the esters and amides of **6a** was not sufficiently rapid to achieve the necessary blood levels of **6a** for good activity.

Amoebiasis. The majority of the compounds in Table I inhibited the growth of *Entamoeba histolytica* in the range 16–64 μg/ml but many showed very poor activity in this test. Table III lists the compounds active against *E. histolytica* in mice and hamsters. The assessment of antiamoebic activity was based on methods described by Jones[12] for intestinal amoebiasis in rats and Reinertson and Thompson[13] for hepatic amoebiasis in hamsters. The parent compound **6a** shows activity approaching the standard compound metronidazole **1**. However, some of the amides exhibit excellent activity against the infections in rats and hamsters. Compounds **22**, **23**, **27**, **28**, and **29** all appear to be more active than metronidazole against intenstinal amoebiasis. None of the compounds were as effective against hepatic amoebiasis in hamsters as metronidazole. The urinary metabolite of **6a** (**11b**) lacked activity against intestinal amoebiasis but still retained activity against the hepatic form of the disease when given ip.

Trypanosomiasis. The primary object of synthesizing **6a** and its derivatives was to determine their activity against trypanosomal infections in mice. Table IV shows that **6a** and its immediate precursors **4**, **5**, and **6b** all show interesting activity against infections of *Trypanosoma rhodesiense*, *Trypanosoma cruzi*, *Trypanosoma gambiense*, and *Trypanosma congolense* in mice when tested using the procedures described by Hawking.[14] The results on the alcohol **6b** suggest that it is more readily absorbed or more rapidly oxidized to **6a** than the aldehyde **5** since it is 2–4 times more active against three out of the four trypanosomal infections. The sodium salt **12** was much less active against *T. rhodesiense* infections than **6a** when given orally and similarly when tested against *T. congolense* ip. We have no explanation for this phenomenon since the acid **6a** and its sodium salt **12** gave identical blood levels when given po or ip to mice.[†]

Replacement of the carboxyl group of biologically active

Table III

Compd	% activity[a] Rats (intest) Dose, mg/kg × 5 po *E. histolytica*	Hamsters (hepatic) Dose, mg/kg × 5 ip *E. histolytica*
4	100 100	100 100
5	100 100	100 Inactive
6b	100 100	100 100
6a	50 100	100 96
12	50 100	100 66
6c	100 Inactive	100 83
13	100 100	100 88
14	100 100	100 67
17	100 100	50 100
20	100 100	100 100
21	100 100	100 50
6g	100 Inactive	100 100
22	12.5 100	100 100
23	12.5 100	100 Inactive
24		25 100
25	25 100	50 100
26	25 100	100 100
27	12.5 100	100 100
28	12.5 100	100 67.5
29	5 100	75 100
31	12.5 Inactive	100 100
6h	100 100	100 100
Metronidazole (**1**)	25 100	25 po 100

[a]Rat: per cent activity is calculated from the extent of visible reduction of pathological change in the caecum together with diminution of parasites present in the caecal lesions. Hamster: per cent activity is calculated from the extent of diminution of liver necrosis together with the reduction of parasites present in the necrotic tissue. In these tests, normal and infected controls were included.

compounds with the comparably acidic 5-tetrazolyl group often, but not always, results in retention of that activity.[15] However, the tetrazole **6d** was barely active against *T. rhodesiense* infections in mice and was inactive against *T. cruzi*.

The nitro compound **6f** was inactive against *T. rhodesiense* and *T. cruzi* while the cyano compound **6c** barely showed activity against *T. rhodesiense* and was inactive against *T. cruzi*. These results suggest that the carboxyl function of **6a** is essential for activity.

The esters **13–20** showed similar activity to the parent acid **6a** against *T. rhodesiense* when dosed ip but were inferior when dosed orally. All the esters except **16** were inactive against *T. cruzi* infections. The amide **21** was inactive against *T. rhodesiense* and *T. cruzi* but showed marginal activity against *T. gambiense* and *T. congolense*. The thioamide **6g** demonstrated a similar pattern of activity.

The secondary amides **23–39** had similar orders of activity against *T. rhodesiense* as the parent acid **6a**, with **22** being the exception. The high activity of **24** against *T. rhodesiense* was somewhat surprising since it would be expected to be metabolized to **11b** and this compound, a urinary metabolite of **6a**, was virtually inactive against that organism.

The tertiary amides did not show a regular pattern of antitrypanosomal activity. Compounds **30** and **34** were as good as the parent acid **6a** but **31**, **32**, and **33** were less active.

Compounds **6a** and **12** were tested against *Trypanosoma vivax* (Desowtiz strain) in mice and both were curative when given at 25 mg/kg × 5 ip or po (experimental conditions were similar to those in Table IV for *T. rhodesiense*). In view of this activity we considered that **6a** or **12** may have useful activity against *T. vivax* and *T. congolense* in cattle.

Table IV. Minimum Dose Level (in mg/kg) 100% Effective Against Trypanosomal Infections in Mice

	T. rhodesiense[a]		*T. cruz*[b]		*T. gambiense*[a]	*T. congolense*[a]
No.	ip	po	ip	po	ip	ip
4	50	100	200	500	25	100
5	25	200	500	500	25	50
6b	25	50	200	200	12.5	100
6a	25	25	100	200	12.5	25.0
12	25	50	100	100	12.5	50
6d	>50	Inactive	Inactive		ND[c]	ND
6c	>200	Inactive	Inactive		ND	ND
6f	Inactive		Inactive		ND	ND
13	50	Inactive	Inactive		50	>200
14	50	200	Inactive		50	200
15	30	50	Inactive		25	50
16	100	50	>100	Inactive	25	100
17	25	Inactive	Inactive		ND	ND
18	25	Inactive	Inactive		ND	ND
19	25	Inactive	Inactive		ND	ND
20	25	Inactive	>50	Inactive	ND	ND
21	Inactive		Inactive		>200	>200
6g	25	Inactive	Inactive		50	>200
22	>50	ND	Inactive		ND	ND
23	25	12.5	100	100	ND	50
24	12.5	12.5	Inactive		ND	ND
25	25	25	>50	ND	ND	ND
26	50	100	200	200	ND	50
27	25	25	50	50	ND	ND
28	50	100	50	>50	ND	25
29	25	25	>50	Inactive	ND	>25
30	50	25	50	>200	ND	12.5
31	Inactive		Inactive		ND	ND
32	100	>200	>200	Inactive	ND	50
33	50	200	100	200	50	100
34	50	25	100	100	25	25
11a	200	ND	Inactive		ND	>100
11b	>50	Inactive	Inactive		ND	ND
6h	50	200	200	500	25	100
Suramin	1	Inactive	Inactive	ND	5	Inactive
Pentamidine	1.25	Inactive	Inactive	ND	5.0	>5
Diminazene	1	Inactive	Inactive	ND	5.0	10
Melarsoprol	0.75	0.5	Inactive at 2	ND	0.75	Inactive

[a]Mice were dosed for four consecutive days, commencing on the day of infection. 100% efficacy is equivalent to 30-day post-infection survival with negative parasitemia. [b]Mice were dosed for five consecutive days commencing on the day of infection. 100% efficacy is equivalent to 60-day post-infection survival with negative parasitemia. [c]ND = not done.

Compound **6a** was tested by Dr. M. Clarkson and Mr. R. Hull of the Liverpool School of Tropical Medicine and Hygiene against a virulent bovine strain of *T. vivax* in calves at a dose level of 25 mg/kg iv given on four consecutive days. The infected control calf died of trypanosomiasis after 35 days, whereas the treated infected animals had a negative parasitaemia after 90 days and were presumed cured. Administration of single iv or im injections of **12** (25 mg/kg) to *T. vivax* infected calves resulted in the calves having a negative plasma parasitaemia 1 day after dosing, but unlike the multiple dosing test, the parasitaemia returned after 8 and 7 days, respectively.

None of the nitroimidazoles described in this paper are as effective against the various trypanosoma as the standard drugs suramin, pentamidine, and diminazene when given parenterally. Several, however, *e.g.*, **6a**, **12**, **23**, and **27**, have a wider range of antiprotozoal activity and are also orally effective. Melarsoprol can be used orally but suffers from the disadvantage of toxicological complications.[1] The overall pattern of antiprotozoal activity demonstrated by the strylimidazoles described in this paper and part I[3] suggests further investigations in this area.

Schistosomiasis. All the compounds were tested against *S. mansoni* infections in mice and activity was assessed using the oogram method described by Pellegrino and coworkers.[16] A number of esters and amides showed marginal activity against the infection in mice (Table V) but none were active against the infection in hamsters.

In conclusion, it appears that **6h** owes its *in vivo* activity to metabolic conversion to **6a** (Tables II–IV). Although conversion of **6a** to various esters and amides results in quantitative differences in biological activity, the overall superiority of **6a** to any of its simple derivatives is evident from the tables.

Experimental Section

Melting points were taken on a Gallenkamp apparatus (Registered Design No. 889339) using capillaries and are uncorrected. All compounds were characterized by ir, uv, nmr, and elemental analyses (C, H, N) which were within ±0.4% of the theoretical values.

2-[4-(1,3-Dioxa-2-cyclopentyl)styryl]-5-nitro-1-vinylimidazole (4). 4-(1,3-Dioxa-2-cyclopentyl)benzaldehyde, 135 g (0.76 mol) (prepared from equimolar proportions of terephthaldehyde and ethanediol by a standard route), and 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole, 85.6 g (0.5 mol), were allowed to react (method B) to give the highly crystalline brownish yellow styryl compound, 53.2 g (32%), which was converted *via* method C to the tosylate, 60.0 g (78%), which in turn was allowed to react *via* method G to give 33.3 g (86%) of the 1-vinyl compound, mp 135–136°. Method B, C, and G were previously described in part I.[3]

2-(4-Formylstyryl)-5-nitro-1-vinylimidazole (5). Compound 4, 49.7 g (0.16 mol), was dissolved in THF (250 ml) by stirring and warming to 40°. H_2O (5 ml) and concentrated HCl (2 ml) were added and stirring was continued for 0.5 hr. After cooling and

Table V. Activity Against *S. Mansoni* in Mice

Compd	Dose level, mg/kg × no. of days	No. of mice in test	No. of mice with oogram change	Comments
18	300 × 5 po + 100 × 5 ip	5	5	>50% mature eggs
19	300 × 5 po + 100 × 5 ip	5	5	<10% stage I and II eggs
20	300 × 5 po + 100 × 5 ip	5	2	>50% mature eggs
23	300 × 5 po + 100 × 5 ip	5	3	>50% mature eggs
28	300 × 5 po + 100 × 5 ip	5	5	>10% dead eggs
29	300 × 5 po + 100 × 5 ip	5	5	>50% mature eggs
Niridazole	50 × 5 ip	5	5	100% dead eggs

standing at room temperature for 1 hr the yellow crystalline solid was collected and recrystallized from EtOAc to give 40.0 g (99%), mp 196–197°.

2-(4-Hydroxymethylstyryl)-5-nitro-1-vinylimidazole (6b). Compound **5**, 17.6 g (0.065 mol), was stirred in *n*-PrOH (100 ml) and cooled to 0°. $NaBH_4$ (2.8 g) in H_2O (25 ml) was added rapidly and the mixture stirred for 1 hr. The solid was collected, washed with H_2O, and recrystallized from EtOAc to afford 12.5 g (71%), mp 204–205°.

2-(4-Carboxystyryl)-5-nitro-1-vinylimidazole (6a). Compound **5**, 80 g (0.3 mol), was stirred in Me_2CO (800 ml) and Jones chromic acid solution (80 ml) was added dropwise over 0.5 hr. Stirring was continued at room temperature for 4 hr. The yellow solid was collected, washed thoroughly with hot H_2O, and dried *in vacuo* at 60°. The crude acid (73.7 g) was suspended in H_2O (1.8 l.) and adjusted to constant pH 9 by the careful addition of 4 *N* NaOH solution. A very fine insoluble yellow solid (unreacted aldehyde, 9.7 g, mp 194–195°) was centrifuged off. The centrifugate was stirred and concentrated HCl added slowly dropwise to pH 3.5. The fine yellow solid was collected, washed with H_2O, recrystallized from dioxane, and dried *in vacuo* at 60° to give 58.9 g (79%) of the acid, mp 306–308° dec.

A portion of **6a** was converted to the Na salt **12** (yield 77%) by dissolving in aqueous solution with the calculated amount of 4 *N* NaOH solution, evaporation *in vacuo* to low volume, addition of 10 volumes of *n*-BuOH, and evaporation *in vacuo* to give yellow crystals which were collected, Me_2CO washed, and dried *in vacuo* at 60°.

2-(4-Cyanostyryl)-5-nitro-1-vinylimidazole (6c). Compound **5**, 26.9 g (0.1 mol), was suspended in EtOH (1 l.) and refluxed for 1 hr with a solution of $NH_2OH \cdot HCl$, 7.0 g (0.1 mol), and NaOAc (16 g) in H_2O (120 ml). After cooling overnight the crystals were collected, H_2O washed, and dried to give 24.5 g (86%) of oxime, mp 231–232°.

The oxime, 14.2 g (0.05 mol), was heated under reflux with Ac_2O (200 ml) for 4 hr. The dark brown solution was poured onto ice (1 kg) and treated with 5 *N* NaOH solution to pH *ca.* 5 to aid hydrolysis of Ac_2O excess. After standing overnight, the brownish yellow solid was collected, H_2O washed, dried, and recrystallized from EtOAc (with C treatment) to give 9.1 g (69%), mp 215–216° dec.

2-(4-Nitrostyryl)-5-nitro-1-vinylimidazole (6f). 5-Nitro-2-styryl-1-vinylimidazole, 4.8 g (0.02 mol), was dissolved with stirring in concentrated H_2SO_4 (*d* 1.84, 25 ml) at 0° and concentrated HNO_3 (*d* 1.5, 1.3 g) was added dropwise. After stirring for 1 hr the clear solution was poured onto ice (250 g) and the resultant bright yellow solid was collected after 1 hr, H_2O washed, dried, and fractionally crystallized from Me_2CO, yield 3.05 g (52%), mp 212–213°.

5-Nitro-2-[4(tetrazol-5-yl)styryl]-1-vinylimidazole (6d). Compound 6c (20.9 g, 0.079 mol), NaN_3 (5.15 g, 0.079 mol), and NH_4Cl (4.3 g, 0.079 mol) were stirred in DMF (140 ml) and gradually brought to reflux (oil bath) and maintained at reflux for 6 hr. The orange brown solution was poured onto ice (1.5 kg) and made alkaline with 5 *N* NaOH solution (small precipitate removed here by filtration). The clear filtrate was carefully acidified to pH 2 with 5 *N* HCl solution and the resultant slimy solid was collected and recrystallized from aqueous MeOH with C treatment to give 3.4 g (14%), mp 203–204° dec.

2-(4-*tert*-Butoxycarbonylmethylcarbamoylstyryl)-5-nitro-1-vinylimidazole (11a). A suspension of **6a** (2.85 g, 0.01 mol) in THF (60 ml) and DMF (10 ml) containing *tert*-butyl glycinate phosphite[17] (2.14 g, 0.01 mol) and TEA (1.1 g, 0.01 mol) was treated with EEDQ (2.47 g, 0.01 mol) and heated under reflux. After 6 hr the clear solution was cooled and poured into H_2O and the resultant solid was extracted with EtOAc. The extract was evaporated and allowed to crystallize, yield 1.4 g (35%), mp 188–190°.

2-(4-Carboxymethylcarbamoylstyryl)-5-nitro-1-vinylimidazole (11b). Compound **11a** (2.8 g, 0.007 mol) was dissolved in TFA (7.5 ml) and stood at room temperature for 0.25 hr. The solution was poured into H_2O (100 ml) and the precipitate was collected and dried. The solid was recrystallized from aqueous DMF to give the acid, 2.1 g (88%), as yellow plates, mp 268–270° dec.

General Method for Esters. The acid chloride of **6a** [yield >95%, mp 180–181°, prepared by refluxing **6a** (100 g, 0.35 mol) with $SOCl_2$ (350 ml) for 2 hr, evaporation of excess $SOCl_2$, C_6H_6 washing, and drying of the crystals] was added with stirring and cooling to an excess of the appropriate alcohol. The mixture was heated on a steam bath for *ca.* 0.5 hr, cooled, and poured into H_2O and the resultant yellow solid was H_2O washed and crystallized from a suitable solvent. Compounds **17**, **18**, and **19** required purification *via* their HCl salts before they readily crystallized. Compounds **15** and **16** were deliberately isolated as the HCl salts to give solids having appreciable solubility in H_2O.

General Method for Amides. The acid chloride of **6a** was added with stirring and cooling to an excess of the appropriate amine. The mixture was stirred at room temperature for 1–2 hr and poured into H_2O and the resultant yellow solid was H_2O washed and crystallized from a suitable solvent, usually EtOAc. In the preparation of **34** the reaction mixture was diluted with dry Et_2O because of the violence of the reaction of the acid chloride with pyrrolidine alone.

5-Nitro-2-(4-thiocarbamoylstyryl)-1-vinylimidazole (6g). A solution of 6c, 8 g (0.03 mol), in DMF–HCl 1/1 complex (80 ml) at 80° was treated with thioacetamide, 4.5 g (0.06 mol), and the mixture heated on a steam bath for 1.5 hr. The yellow crystalline slurry was poured onto ice (500 g), the solid was collected, H_2O washed, and extracted with boiling EtOH, and the residual solid was crystallized from aqueous DMF, yield 8.2 g (90%), mp 218–220°.

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Isolation, X-Ray Analysis, and Synthesis of a Metabolite of (−)-3-Hydroxy-*N*-allylmorphinan

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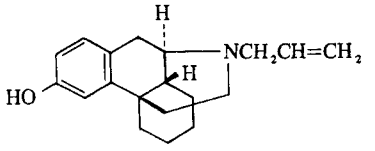
The structure of a metabolite (2) of (−)-3-hydroxy-*N*-allylmorphinan (1, levallorphan) isolated from urine of rats was established by single-crystal X-ray analysis of the HBr salt to be (−)-*N*-allyl-3,6β-dihydroxymorphinan (2). Compound 2 was synthesized from (−)-3-methoxy-6-oxo-*N*-methylmorphinan (3). No analgesia was observed for 1 or 2 in the tail flick, hot plate, and Nilsen tests. The two compounds were approximately equal in their antagonism to morphine in the tail flick and Nilsen methods.

Previous studies[1] on the *in vivo* and *in vitro* metabolism of levallorphan (**1**), a potent morphine antagonist, demonstrated the formation of two metabolites. One metabolite (metabolite II) was found to be identical with (−)-3-hydroxymorphinan. The other metabolite (metabolite I) was isolated from rat urine and rat liver incubation mixtures, but the structure was not elucidated.



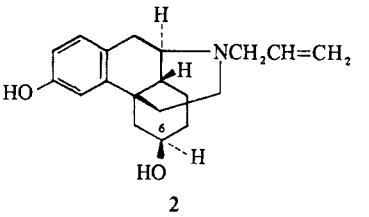
1

Elemental analysis[1] and mass spectral data† indicated that metabolite I had been formed by the addition of one oxygen to levallorphan (**1**). Chemical and spectral studies were unable to ascertain the exact position of the oxygen.

For further characterization of this metabolite, urine from rats treated with 17.6 g of levallorphan tartrate was collected. After hydrolysis of the urine with HCl, the metabolite was isolated by a series of extractions and column chromatography procedures described in the Experimental Section. After repeated crystallizations, 33 mg of crystals was obtained with a melting point which compared favorably to that reported for sublimed metabolite I.[1]

A single-crystal X-ray analysis of **2**·HBr revealed that **1** had been oxidized at the 6β position. The structure and configuration of the metabolite are shown in the stereodrawing (Figure 1).

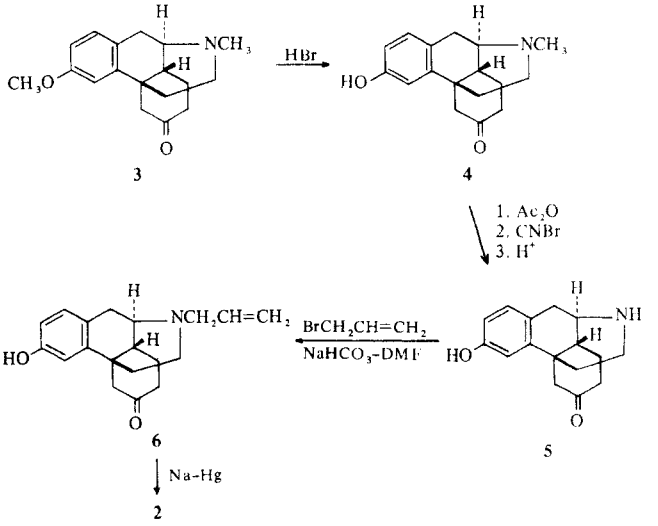
For comparison of the biological activity of **1** and **2**, compound **2** was synthesized according to Scheme I. Treatment of **3** with 48% hydrobromic acid at reflux temperature gave the phenol **4**. The *O*-acetyl derivative of **4** on treatment with cyanogen bromide in chloroform yielded, after acid hydrolysis, the secondary amine **5**. Alkylation of **5** with allyl bromide in dimethylformamide in the presence of sodium bicarbonate gave the *N*-allylmorphinan **6**. Sodium amalgam reduction of **6** afforded the desired 6β-alcohol **2**, which was purified by fractional crystallization. The nmr spectrum of the crude reduction product indicated the presence of a minor amount of the epimeric 6α-alcohol. No attempt was made to isolate the epimer.



2

The mass spectrum of synthetic **2** shows the molecular ion as required at *m/e* 299. The nmr spectrum features a

Scheme I



†H. M. Fales, unpublished results.