Schistosomicides. 2.[1] Derivatives of 2,3,4,4a,5,6-Hexahydro-1*H*-pyrazino[1,2-*a*]quinoline

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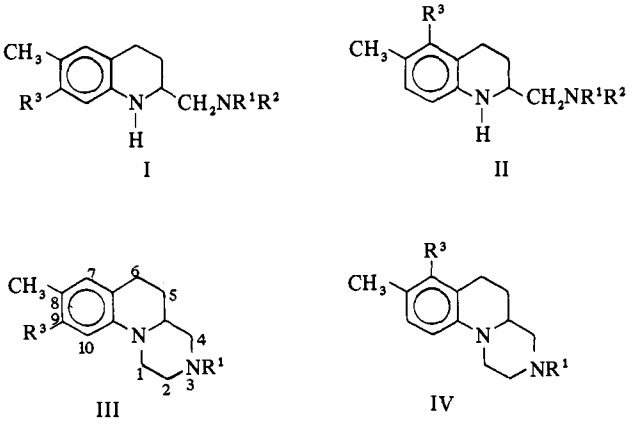
The synthesis and schistosomicidal activity of novel 2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]quinolines, III, are described. In the 9-NO_2 series (III; $R^3 = NO_2$), highest activity is displayed when R^1 = a 3-C substituent, *e.g.*, Pr, *i*-Pr, allyl, or acetonyl, whereas in the 9-Cl series (III; R_3 = Cl) the most active compound is the parent member **25** (III; R^1 = H; R^3 = Cl). Activity is enhanced by the introduction of a 10-Me group in the latter series, which may be a consequence of a change in molecular conformation. Activity is also displayed by isomeric compounds IV. The most active compound in the group is **35**, 9-chloro-8,10-dimethyl-2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]quinoline. Several 8-hydroxymethyl analogs of series III, prepared by means of a microbial hydroxylation procedure, show enhanced activity, and a compound of particular interest is **46** (derived from **35**), which is curative against *Schistosoma mansoni* in monkey at single im doses of 2.5 to 5.0 mg/kg.

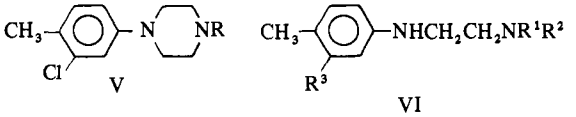
Part 1 of this series[1] described the synthesis and schistosomicidal activity of a novel series of 2-aminomethyl-1,2,-3,4-tetrahydroquinolines (I), a compound of particular interest being 2-*N*-isopropylaminomethyl-6-methyl-7-nitro-1,2,3,4-tetrahydroquinoline [I, R^1 = H, R^2 = $CH(CH_3)_2$; R^3 = NO_2], which was highly active in single oral dose against *Schistosoma mansoni* infections in mice and in monkeys. As an extension of this structural type, which displays a degree of conformational constraint, we have investigated the comparatively more rigid 2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]quinoline derivatives (III). Isomeric compounds of type IV were also investigated, despite the finding[1] that 1,2,3,4-tetrahydroquinolines (II) bearing an electronegative substituent (R^3) in the 5 position, were devoid of schistosomicidal activity.



I II III IV

R^1 = H, alkyl; R^2 = alkyl; R^3 = halogen, NO_2

Compounds of types III and IV also incorporate the structural features of the *N*-phenylpiperazine series[2] V, derived from the mirasan series[3] VI, of which VI (R^1 = R^2 = C_2H_5; R^3 = Cl)† is the parent member.



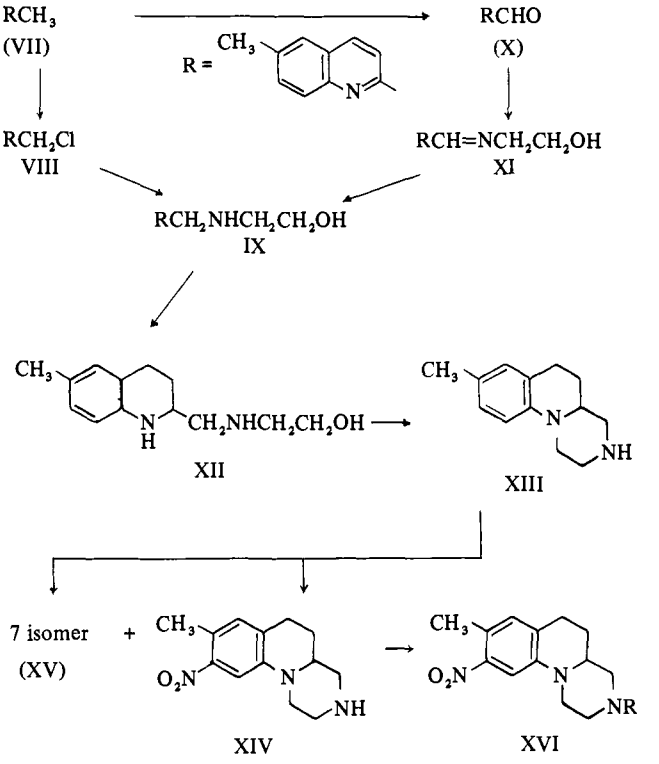
V VI

As in the case of compounds I a prime objective of the program was the development of agents which, unlike members of series V and VI, would display worthwhile activity against schistosomes in primates.[1]

†Mirasan

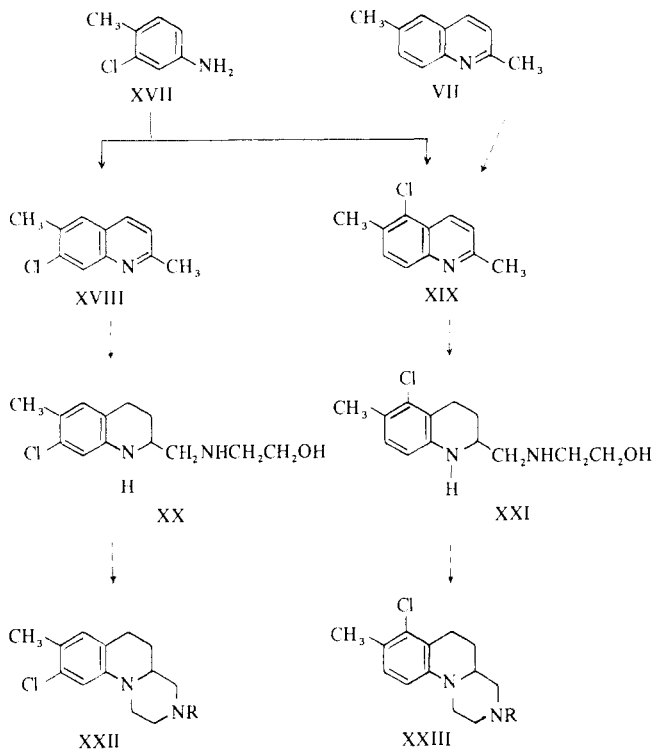
Chemistry. A novel synthetic route was devised for obtaining the 2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]quinoline ring system which appears to offer advantages over alternative published methods[4-8] in terms of versatility, overall reaction yields, and/or freedom of side reactions. A general scheme for NO_2 derivatives is indicated in Scheme I.

Scheme I. General Reaction Scheme for Nitro Compounds

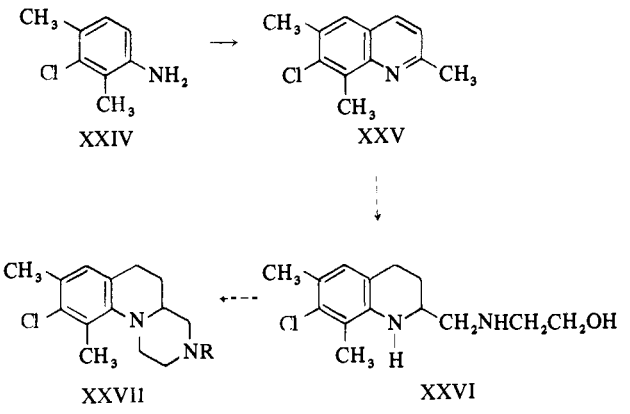


The key intermediate, 2-(*N*-2-hydroxyethylaminomethyl)-6-methylquinoline (IX) was obtained from 2,6-dimethylquinoline (VII) either (a) by selective monochlorination[9] with Cl_2 in CCl_4 containing Na_2CO_3 and treating the product, 2-chloromethyl-6-methylquinoline (VIII), with ethanolamine, or (b) by condensing 2-formyl-6-methylquinoline[10] (X) with ethanolamine and reducing the Schiff base with $NaBH_4$. The amine IX was hydrogenated to the corresponding 1,2,3,4-tetrahydroquinoline (XII) over Raney Ni; alternatively XII was obtainable by Pt-catalyzed double hydrogenation of the Schiff base, XI. Cyclization of XII was achieved with P_2O_5 in xylene and nitration of the product, **XIII**, in H_2SO_4 afforded **XIV** and a trace of the 7-NO_2 iso-

Scheme II. General Reaction Scheme for Chloro Compounds



Scheme III. Reaction Scheme for 10-Methyl Chloro Compounds



mer which were readily separable by fractional crystallization.‡ The derivatives listed in Table I were obtained by alkylation of XIV (**1**) in the presence of K_2CO_3, or by methods indicated in the Table.

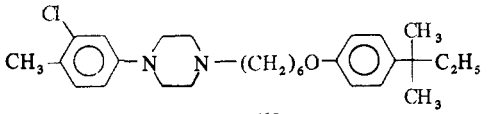
Methods for the preparation of chloro members in the series are outlined in Schemes II and III. The 5- and 7-chloroquinolines, XIX and XVIII, respectively, were obtained by fractional distillation of the Doebner-Miller reaction product from 3-chloro-4-methylaniline (XVII) and the 2-Me group was functionalized by methods outlined in Scheme I. Compound XIX was also obtainable[11] from 2,6-dimethylquinoline (VII) by nitration, which occurs exclusively in the 5 position, followed by reduction with $SnCl_2$, diazotization of the 5-amino compound, and Sandmeyer reaction. Cyclization of XX and XXI was accomplished with P_2O_5 in xylene (yields were improved by including "hyflo" as dispersant, *cf.* ref 12) or polyphosphoric ester, and alkylation of the products gave 2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]quinolines, XXII and XXIII, respectively.

Compounds XXVII (Scheme III) bearing a Me group in the 10 position, were prepared similarly starting from 3-chloro-2,4-dimethylaniline (XXIV). One advantage of this series was the avoidance of the isomer problem at the quinaldine synthesis stage.

Screening Methods. Primary Screening. Primary screening was undertaken in mice infected with an East African strain of *Schistosoma mansoni* and compounds in the form of their free bases or a salt derivative (*e.g.*, hydrochloride, *p*-toluenesulfonate, or hydrogen maleate) were administered orally as a single dose of 12.5 or 50 mg/kg (base equiv). Activity was assessed[13] by the hepatic shift method (the movement of adult worms from the mesenteric plexus to the intrahepatic vessels) 96 hr after administration of the compound.

Secondary Screening. Secondary evaluation was undertaken in vervet monkeys *(Cercopithecus aethiops)* infected with the same strain of *S. mansoni* employed in rodents. Fecal egg output (initially 5000-10,000/24 hr) was determined[14] daily before and after treatment, and efficacy was judged by the reduction in egg load. Treatment was claimed as curative when the count fell to zero and remained so for several weeks.

Schistosomicidal Activity in Mice. Structure-Activity Relationships. The 9-Nitro Series (XVI). The effect of substitution at position 3 is indicated in Table I. Highest activity in the *N*-alkyl series was displayed by members bearing a 3-C substituent, *i.e.*, with a Pr, *i*-Pr, allyl, or acetonyl group (**4**, **8**, **11**, **12**); many derivatives of **12**, such as the oxime **13** proved to be inactive. The reduced activity displayed by higher homologs (**5**, **6**, **7**), branched-chain derivatives (**9**, **10**), and compounds with bulky substituents (**15**, **16**) suggests that there is a critical steric factor operative at this position. The usual effect of introducing into the side chain groups such as CN (**17**), OH (**18**), NEt_2 (**19**), and CO_2Et (**20**), was to reduce activity. Acylation (**22**), sulfonylation (**23**), or thiocarbamoylation (**24**) also resulted in loss of activity, which is in keeping with the requirement[1] for a basic distal N. The synthesis of **21** (and **34** and **45** in Tables II and III, respectively) was prompted by the activity reported[16] for the Abbott compound XXVIII, but no activity was detected for **21** (or **34** and **45**) at single or multiple doses.



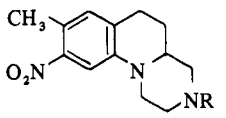
XXVIII

The 9-Chloro Series (XXII). As these compounds proved to be highly potent, assessment of schistosomicidal effects was made after administering a single oral dose of 12.5 mg/kg, *i.e.*, 0.25 the dose level used for the 9-NO_2 series (XVI).

As seen in Table II, the effect of N-alkylation in the 9-Cl series was to reduce activity, in marked contrast to the situation with the analogous 9-NO_2 series (XVI). One might have predicted this general difference between series XVI and XXII, by applying arguments based on the relative lipophilic character of substituents. Assuming the validity of the principle relating to the additivity of substituent π values for estimating molecular lipophilicity,[17] and assuming that molecular lipophilicity is an important factor in determining drug activity,[18] it may be reasoned that a molecule

‡ The identification of each isomer was readily made by means of its nmr spectrum, XIV showing two singlets and XV showing a quartet in the aromatic region; *cf.* ref 1.

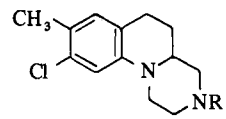
Table I



Compound	R	Oral activity[a] at 50 mg/kg (base equiv) X 1	Salt	Mp, °C	Formula[g]	Method of synthesis[b]
1	H	++	Maleate	203-204	$C_{13}H_{17}N_3O_2 \cdot C_4H_4O_4$	A
2	CH_3	+	Maleate	182-185	$C_{14}H_{19}N_3O_2 \cdot C_4H_4O_4$	B
3	C_2H_5	++	Maleate	172-173	$C_{15}H_{21}N_3O_2 \cdot C_4H_4O_4$	B
4	$(CH_2)_2CH_3$	+++	Maleate	169-170	$C_{16}H_{23}N_3O_2 \cdot C_4H_4O_4$	B
5	$(CH_2)_3CH_3$	++	Maleate	183-184	$C_{17}H_{25}N_3O_2 \cdot C_4H_4O_4$	B
6	$(CH_2)_4CH_3$	+	Maleate	189-190	$C_{18}H_{27}N_3O_2 \cdot C_4H_4O_4$[c]	B
7	$(CH_2)_6CH_3$	-	Maleate	168-172	$C_{20}H_{31}N_3O_2 \cdot C_4H_4O_4$	B
8	$CH(CH_3)_2$	+++	Maleate	196	$C_{16}H_{23}N_3O_2 \cdot C_4H_4O_4$	B
9	$CH_2CH(CH_3)_2$	-	Maleate	209-210	$C_{17}H_{25}N_3O_2 \cdot C_4H_4O_4$	B
10	$CH_2CH_2CH(CH_3)_2$	-	Maleate	213-214	$C_{18}H_{27}N_3O_2 \cdot C_4H_4O_4$	B
11	$CH_2CH{=}CH_2$	+++	Base	108	$C_{16}H_{21}N_3O_2$[d]	B
12	CH_2COCH_3	+++	HCl	>300	$C_{16}H_{21}N_3O_3 \cdot HCl$	B
13	$CH_2C{=}NOH \cdot CH_3$	-	Base	164-166	$C_{16}H_{22}N_4O_3$	C
14	$CH_2CHOH \cdot CH_3$	++	HCl	264-267	$C_{16}H_{23}N_3O_3 \cdot HCl$	B
15	CH_2COPh	-	Base	130-133	$C_{21}H_{23}N_3O_3$	B
16	CH_2Ph	-	Maleate	220-222	$C_{20}H_{23}N_3O_2 \cdot C_4H_4O_4$	B
17	CH_2CH_2CN	-	Base	111-112	$C_{16}H_{20}N_4O_2$	D
18	CH_2CH_2OH	-	Maleate	125-126	$C_{15}H_{21}N_3O_3 \cdot C_4H_4O_4$	B
19	$CH_2CH_2N(C_2H_5)_2$	-	Dimaleate	130-133	$C_{19}H_{30}N_4O_2 \cdot 2C_4H_4O_4$[e]	B
20	$CH_2CO_2C_2H_5$	-	Base	91-94	$C_{17}H_{23}N_3O_4$[f]	B
21	$(CH_2)_6O$–C$_6$H$_4$–$C(CH_3)_3$	-	Maleate	192-193	$C_{29}H_{41}N_3O_3 \cdot C_4H_4O_4$	B
22	$COCH_3$	-	Base	137-139	$C_{15}H_{19}N_3O_3$	E
23	SO_2Ph	-	Base	194-196	$C_{19}H_{21}N_3O_4S$	F
24	$CS \cdot NHPh$	-	Base	165-166	$C_{20}H_{22}N_4O_2S$	G

[a]Activity rating +++, hepatic shift >60%; ++, 30-60%; +, <30%; -, nil, against *S. mansoni* in mice; in the case of inactive compounds, activity was often displayed at higher dose levels. [b]A, by methods outlined in Scheme I. B, by alkylation of 1. C, oximation of 12. D, cyanoethylation of 1, *cf.* ref 15. E, reaction of 1 with acetic anhydride. F, reaction of 1 with phenylsulfonyl chloride. G, reaction of 1 with phenyl isothiocyanate. [c]C: calcd, 60.95; found 60.40. [d]H: calcd, 7.37; found 6.94. [e]C: calcd, 56.04; found 56.52. [f]H: calcd; 6.95; found 6.51%. [g]All compounds were analyzed for C, H, N.

Table II.



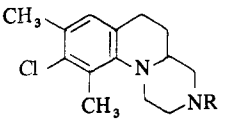
Compound	R	Oral activity[a] @ 12.5 mg/kg (base equiv) X 1	Salt	Mp, °C	Formula[c]
25	H	++	HCl	>300	$C_{13}H_{17}ClN_2 \cdot HCl$
26	CH_3	++	Maleate	170-171	$C_{14}H_{19}ClN_2 \cdot C_4H_4O_4$
27	C_2H_5	+	Maleate	149-150	$C_{15}H_{21}ClN_2 \cdot C_4H_4O_4$
28	$(CH_2)_2CH_3$	+	Maleate	149-150	$C_{16}H_{23}ClN_2 \cdot C_4H_4O_4$
29	$(CH_2)_3CH_3$	-	Maleate	162-164	$C_{17}H_{25}ClN_2 \cdot C_4H_4O_4$
30	$CH(CH_3)_2$	+	Maleate	190-191	$C_{16}H_{23}ClN_2 \cdot C_4H_4O_4$[b]
31	$CH_2CH{=}CH_2$	+	Base	75	$C_{16}H_{21}ClN_2$
32	CH_2COCH_3	+	Maleate	148-150	$C_{16}H_{21}ClN_2O \cdot C_4H_4O_4$
33	CH_2CH_2OH	-	Maleate	150-153	$C_{15}H_{21}ClN_2O \cdot C_4H_4O_4$
34	$(CH_2)_6O$–C$_6$H$_4$–$C(CH_3)_3$	-	Maleate	177	$C_{29}H_{41}ClN_2O \cdot C_4H_4O_4$

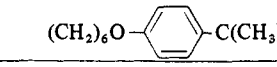
[a]Activity rating as in Table I; N. B. reduction in dose. [b]N: calcd, 7.10; found 6.68%. [c]See Table I, footnote *g*.

such as **25**, containing a Cl atom (π value 0.9), would show similar activity to a molecule with a corresponding 9-NO_2 group (π value 0.49) and bearing an *N*-alkyl substituent with a π value of approximately 0.4, *i.e.*, a Me group (π value 0.5). However, as previously mentioned, the most active members of series XVI were those containing higher alkyl groups (**4**, **8**, and **11**) with π values of 1.2 to 1.5 and, perhaps surprisingly, **12**, bearing an acetonyl group with a π value of −0.21. Furthermore, all of these 9-NO_2 compounds were considerably less active than **25**, the most active of series XXII. It is clear therefore, that whereas a consideration of lipophilicity factors[1] may account for certain biological differences between the mirasan series (VI) and the tetrahydroquinolines (I), the present case with pyrazinoquinolines is more complex.

Regarding the relative effects of Cl and NO_2 substituents

Table III.



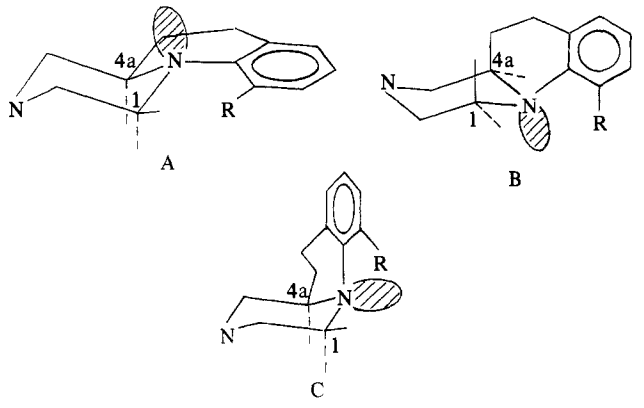
Compound	R	Oral activity[a] @ 12.5 mg/kg (base equiv) X 1	Salt	Mp, °C	Formula[b]
35	H	+++	Base	94-96	$C_{14}H_{19}ClN_2$
36	CH_3	++	Maleate	103	$C_{15}H_{21}ClN_2 \cdot C_4H_4O_4$
37	CH_2CH_3	++	Maleate	138	$C_{16}H_{23}ClN_2 \cdot C_4H_4O_4$
38	$CH_2CH_2CH_3$	++	Maleate	125-126	$C_{17}H_{25}ClN_2 \cdot C_4H_4O_4$
39	$(CH_2)_3CH_3$	-	Maleate	139-140	$C_{18}H_{27}ClN_2 \cdot C_4H_4O_4$
40	$CH(CH_3)_2$	++	Maleate	174-175	$C_{17}H_{25}ClN_2 \cdot C_4H_4O_4$
41	$CH_2CH{=}CH_2$	++	Base	67	$C_{17}H_{23}ClN_2$[c]
42	CH_2COCH_3	++	HCl	250	$C_{17}H_{23}ClN_2O \cdot HCl$
43	$CH_2CHOH \cdot CH_3$	-	Base	144-147	$C_{17}H_{24}ClN_3O$
44	CH_2CH_2OH	++	Maleate	132	$C_{16}H_{23}ClN_2O \cdot C_4H_4O_4$
45		-	Maleate	159	$C_{30}H_{43}ClN_2O \cdot C_4H_4O_4$

[a] Activity rating as in Table I; N. B. reduction in dose. [b] See Table I, footnote g. [c] N anal. only.

on activity, it is of interest to recall that the tetrahydroquinoline series (I) is unique among this general class of schistosomicides in that the 7-NO_2 members are *more* active than the corresponding 7-Cl compound.[1]

The 9-Chloro-10-methyl Series (XXVII). Activity of this series is shown in Table III. Structure-activity relationships were similar to those of the 9-Cl series (XXII), *i.e.*, N-alkylation resulted in a reduction in activity. However, the activity displayed by members of this series was much higher, usually up to twice that displayed by the corresponding members of series **XXII**; the most active compound was **35**, which produced an hepatic shift of 68% at a single oral dose of 12.5 mg/kg.

It has been shown previously[1] that the schistosomicidal activity in series I is governed by the stereochemistry of the side chain. It was therefore considered that the enhanced activity of series XXVII compared with XXII could be due to the geometry of the fused piperazine ring being favorably influenced by the 10-Me group. It is presumed that the favored conformations of the piperazine ring and pyridine ring in the pyrazinoquinoline system are chair and half-chair conformations, respectively.[19,20] For each enantiomer (optical isomerism results from the asymmetric C at position 4a) equilibrium between 3 extreme forms may in principle exist, as depicted in diagram 1 (substituents at positions 3, 8, and 9 have been omitted since they are superfluous to the argument). The fusions of the heterocyclic rings in A are trans (axial N lone pair; axial 4a proton), in B are cis (axial lone pair; equatorial 4a proton), and in C are also cis (equatorial lone pair; axial 4a proton).



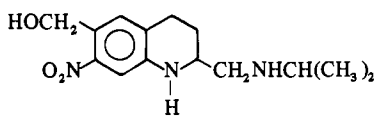
The conformation of heterocyclic systems containing bridgehead N atoms has been the subject of recent study, involving both ir[19,21] and nmr[22,23] techniques. Comparison of the ir spectra for analogous pairs of compounds in series XXII and XXVII for presence or absence of Bohlmann bands[24] in the 2700-2800 cm^{-1} region was not considered a reliable method because of the ambiguously low intensity absorptions encountered. However, a significant difference was found in the nmr spectra for all compounds examined. Members of series XXII showed a signal at δ 3.6 (1 proton multiplet) attributed to the 4a proton, but nothing above 3.3 was observed for series XXVII. Since the shielding effect of the N lone pair on the 4a proton is comparatively less in a trans conformation than a cis conformation it is assumed that the planar conformation A is preferred for series XXII. Presumably for series XXVII, the "twist" conformation B is preferred over the "bent" conformation C since Dreiding models show considerably less nonbonded interaction between the 10-Me protons and those at position 1.

7-Chloro and 7-Nitro Series (XXIII and XV). These two series were not investigated in detail since they were chemically less accessible than the isomeric 9-substituted series. However, high activity was displayed and the "parent" member of series XXIII (R = H) showed the same level of activity as **25**, the corresponding member of series XXII (R = H). This finding was in marked contrast to the situation with the tetrahydroquinoline isomeric series I and II, when it was found that the 5-substituted series II was devoid of activity.

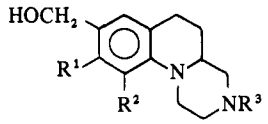
8-Hydroxymethyl Derivatives. As previously mentioned,[1] members of series I are metabolized in a variety of animal species to the corresponding 6-hydroxymethyl derivatives. These metabolites, which may be prepared conveniently in quantity by an oxidative fermentation technique using the "parent" 6-Me compound as substrate in the presence of a strain of *Aspergillus sclerotiorum* Huber,§ have been shown[1] to be highly schistosomicidal, one of the most active compounds being 6-hydroxymethyl-2-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline (XXIX).

Using the above microbiological hydroxylation procedure, several members of the pyrazinoquinoline group have been converted[25] to the corresponding 8-hydroxymethyl deriva-

§ Obtained from the Central bureau voor Schimmelcultures, Baarn, Netherlands (No. 549.65).



XXIX



XXX

tives of the type XXX (R^1 = Cl or NO_2; R^2 = H or CH_3; R^3 = H or alkyl). Many of these compounds proved to be highly schistosomicidal and in summary, the most active was 9-chloro-8-hydroxymethyl-10-methyl-2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]quinoline (**46**; XXX, R^1 = Cl; R^2 = CH_3; R^3 = H), derived from **25**. In mice, **46** displayed approximately twice the activity of XXIX, the most active of the prototype tetrahydroquinoline series, after a single oral dose.

Schistosomicidal Activity in Monkey. A prime objective of the program was to establish whether the pyrazinoquinolines displayed worthwhile activity in primates and assessment in infected monkeys was undertaken.

Several members of the series proved to be highly effective in reducing fecal egg counts, following a single dose schedule. Although structure-activity patterns roughly paralleled those in mice, there were occasional exceptions. Thus, **40** proved to be somewhat more effective than **35** (similar egg count reductions followed a single oral dose administration of 50 mg/kg and 75 mg/kg, respectively).

8-Hydroxymethyl analogs of type XXX were extremely effective, particularly by the im route, one outstanding compound in this class being **46**, which was curative in monkeys at 2.5 to 5 mg/kg × 1, im. This represents a 2-fold enhancement in potency previously reported[1] for XXIX.

Thus, we are encouraged by these results to believe that agents from this chemical group would be effective as schistosomicides in human therapy.

Experimental Section

Melting points were obtained on an Electrothermal melting point apparatus and are corrected. Pmr spectra were recorded on a Varian A60 spectrometer (TMS as internal standard) using $CDCl_3$ as solvent. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

Nitro Compounds. 2-(β-Hydroxyethylaminomethyl)-6-methyl-1,2,3,4-tetrahydroquinoline (XII). (a) *Via* **2-(β-Hydroxyethylaminomethyl)-6-methylquinoline (IX).** 2-Chloromethyl-6-methylquinoline[1] (1.2 kg) was added to a stirred soln of ethanolamine (6.15 l.) in EtOH (6.72 l) at 60–70° and the mixt set aside overnight. The mixt was evapd *in vacuo*, the residue poured into H_2O (10 l.), and the oil extd with $CHCl_3$ (2 × 2 l); the combined $CHCl_3$ exts were washed with H_2O, dried ($MgSO_4$), and evapd to yield a dark brown oil (1.24 kg; 91.5%). A portion was crystd from $CHCl_3$–petr ether (bp 60–80°) to give white needles, mp 74–75°. *Anal.* ($C_{13}H_{16}N_2O$) C, H, N.

The above quinoline (5.9 g) in EtOH (100 ml) was hydrogd at 75° for 7 hr over Wl Raney Ni (5 ml) at an initial H_2 pressure of 52.73 kg/cm². The catalyst was filtered, the EtOH evapd *in vacuo*, and the residue recrystd from EtOAc to give colorless plates (4.3 g; 71.5%), mp 88°. *Anal.* ($C_{13}H_{20}N_2O$) C, H, N.

(b) *Via* **2-(β-Hydroxyethyliminomethyl)-6-methylquinoline (XI).** Ethanolamine (15 g) was cautiously added to a warm soln of 2-formyl-6-methylquinoline[1] (42 g) in EtOH (200 ml) and the mixt refluxed for 2 hr. The cooled soln deposited white needles (48.6 g; 92.5%), mp 147–149°. *Anal.* ($C_{13}H_{14}N_2O$) C, H, N.

The above Schiff base (45 g) in EtOH (500 ml) was hydrogd over Raney Ni (5 g) at 75° for 7 hr at an initial H_2 pressure of 52.73 kg/cm². The catalyst was filtered and the filtrate evapd *in vacuo* to give a yellow oil which solidified on standing. Recrystn from EtOAc gave a white solid (32.5 g; 70%), mp 88–88.5°, identical with sample prepd by method a.

8-Methyl-2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]quinoline (XIII). A mixt of XII (23.4 g) and P_2O_5 (48 g) of dry xylene (250 ml) was stirred while the temp was raised to 135° over 2.5 hr, and finally refluxed at this temp for 24 hr. The solvent was evapd *in vacuo* and the residue extd thoroughly with 5 *N* HCl; this acid ext was basified by addn of 5 *N* NaOH, the mixt extd into Et_2O and the dried ($MgSO_4$) Et_2O exts evapd *in vacuo* to yield a dark oil which was fractionally distd to yield a colorless oil (13.3 g; 62%), bp 122–124° (0.25 mm). A sample was treated with maleic acid in EtOAc to yield the maleate salt, mp 157–158°. *Anal.* ($C_{13}H_{18}N_2 \cdot C_4H_4O_4$) C, H, N.

8-Methyl-9-nitro-2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]quinoline (XIV). A soln of HNO_3 (specific gravity 1.5) (4.86 g) in H_2SO_4 (27 ml) was added over 1 hr to an ice-cooled stirred soln of XIII (22.82 g) in H_2SO_4 (160 ml) and the resulting dark soln stirred at 0–5° for a further 4.5 hr. After this time the mixt was added to ice–H_2O, the resulting soln basified with K_2CO_3, and the oily product extd with $CHCl_3$. The dried ($MgSO_4$) $CHCl_3$ ext was evapd *in vacuo*, and the oily residue was dissolved in EtOAc (50 ml) and treated with a soln of maleic acid (26.2 g) in EtOAc (250 ml). The pptd solid was recrystd from MeOH to yield golden yellow plates (22.1 g; 62.5%), mp 203–204°. *Anal.* ($C_{13}H_{17}N_3O_2 \cdot C_4H_4O_4$) C, H, N.

8-Methyl-7-nitro-2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]quinoline (XV, R = H). Mother liquors from the prepn of XIV were found to contain 70% of the 7-NO_2 isomer by glc analysis. Recrystn of this free base mixt from petr ether (bp 80–100°) yielded a red oil and a yellow solid. The solid was sepd and recrystd from the same solvent to yield the product as a yellow powder, mp 109–111°. *Anal.* ($C_{13}H_{17}N_3O_2$) C, H, N.

3-Allyl-8-methyl-9-nitro-2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]quinoline (11). This preparative procedure typifies that used for alkyl derivatives listed in Tables I, II, and III. A mixt of XIV (5.0 g), allyl chloride (3.12 g), and K_2CO_3 (5 g) in dry MeOH (100 ml) was stirred and refluxed for 20 hr. The cooled mixt was filtered, the MeOH evapd *in vacuo* and the residue treated with H_2O and Et_2O. The Et_2O ext was sepd, dried ($MgSO_4$), and evapd *in vacuo* to give a brown solid which recrystd from EtOH as an orange powder (3.2 g; 55%), mp 108°. *Anal.* ($C_{16}H_{21}N_3O_2$) C, H, N.

Chloro Compounds. 7-Chloro-2-(β-hydroxyethylaminomethyl)-6-methyl-1,2,3,4-tetrahydroquinoline (XX). A mixt of 7-chloro-2-formyl-6-methylquinoline[1] (4.08 g) and ethanolamine (1.22 g) in EtOH (75 ml) was warmed for 90 min and dild to 250 ml with EtOH and this soln hydrogd over prehydrogd PtO_2 (0.5 g), at an initial H_2 pressure at 3.5 kg/cm². A mixt of AcOH (5 ml) and EtOH (5 ml) was added and hydrogn continued. When hydrogn was complete the catalyst was filtered, the soln was concd and poured into H_2O (200 ml), and 5 *N* NaOH was added until basic. The organic product was extd into $CHCl_3$ and evapn of the dried ($MgSO_4$) $CHCl_3$ ext gave a solid (3.5 g; 69.2%), recrystd from EtOAc, mp 92–93°. *Anal.* ($C_{13}H_{19}ClN_2O$) C, H, N.

9-Chloro-8-methyl-2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]quinoline (XXII; R = H). A mixt of XX (3.5 g) in polyphosphoric ester[26] (350 g) was stirred and heated at 130–140° for 30 min. The product was poured into H_2O, the mixt basified with KOH and the whole extd with $CHCl_3$. The dried ($MgSO_4$) $CHCl_3$ ext was evapd *in vacuo*, the resulting dark yellow oil treated with 5 *N* HCl (140 ml) and the mixt heated on the steam bath with the addn of hot H_2O, until dissolution occurred. The solid pptd on cooling was filtered, dried, and recrystd from MeOH to yield white crystals of the HCl salt (20.7 g; 55.2%), mp >300°. *Anal.* ($C_{13}H_{17}ClN_2 \cdot HCl$) C, H, N.

7-Chloro-6,8-dimethyl-2-(β-hydroxyethyliminomethyl)quinoline. A soln of ethanolamine (2.4 g) in EtOH (20 ml) was added to a hot soln of 7-chloro-6,8-dimethyl-2-formylquinoline[1] (8.0 g) in EtOH (500 ml) and the resulting dark soln heated on the steam bath for 0.5 hr. The cooled reaction mixt deposited an off-white solid which recrystd from MeOH as plates (6.8 g; 71%), mp 122–123°. *Anal.* ($C_{14}H_{15}ClN_2O$) C, H, N.

7-Chloro-6,8-dimethyl-2-(β-hydroxyethylaminomethyl)-1,2,3,4-tetrahydroquinoline (XXVI). A soln of the above Schiff base (2.0 g) in EtOH (150 ml) was hydrogd over Raney Ni (1 ml) at 75° and an initial H_2 pressure of 52.73 kg/cm² for 0.75 hr. The catalyst was filtered and evapn of the solvent yielded a solid which was recrystd from MeOH to give white needles (1.2 g; 58.7%), mp 109–111°. *Anal.* ($C_{14}H_{21}ClN_2O$) C, H, N.

9-Chloro-8,10-dimethyl-2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]quinoline (XXVII; R = H). A hot soln of XXVI (3.2 g) in xylene (25 ml) was added to a stirred warm suspension of P_2O_5 (6.4 g) and hyflo (3.2 g) in xylene (100 ml), and the resulting mixt stirred and refluxed for 20 hr. The cooled reactn was made acid by the addn of 5 *N* HCl, the reactn filtered, and the xylene extd with Et_2O. The aqueous acid ext was basified with K_2CO_3, the soln extd with $CHCl_3$, and the dried ($MgSO_4$) $CHCl_3$ ext evapd *in vacuo* to

give a mobile yellow oil (2.0 g) which solidified on standing. The crude solid was recrystd from petr ether (bp 40–60°) to yield a white powder (1.2 g; 40.2%), mp 94–96°. *Anal.* ($C_{14}H_{19}ClN_2$) C, H, N.

Acknowledgments. We thank Dr. E. R. H. Jones for his encouragement and advice during the course of these investigations, Dr. R. Foster and his staff for the biological evaluations, Mr. R. F. Chambers, Mr. J. Graves, and Mr. P. Sherrington for excellent technical assistance, Mr. G. F. Parker and his colleagues for the fermentation aspects of the work, Dr. I. Appleby and his staff for process improvements, and Dr. M. J. Sewell and his staff for analytical services.

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Structure of Hydroxycotinine, a Nicotine Metabolite†

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Received October 13, 1971

In an attempt to establish the structure of hydroxycotinine, a mammalian metabolite of nicotine isolated from the urine of smokers, the syntheses of both diastereomeric 3-hydroxycotinines were undertaken. Two independent routes led to the same 3-hydroxycotinine which upon epimerization gave metabolic hydroxycotinine. Mass spectral and nmr analyses of these diastereomers and also deuterated model compounds established the structure of metabolic hydroxycotinine to be *trans*-1-methyl-3-(*R*)-hydroxy-5-(*S*)-3-pyridyl-2-pyrrolidinone.

The major metabolic pathways of the tobacco alkaloid nicotine (**1**) in the mammalian species studied involve a series of oxidations of the pyrrolidine ring[1] to produce in general more polar and pharmacologically less active[2] compounds than the parent substance. The γ-lactam cotinine (**2**) is the principal metabolite of nicotine and has been reported to be further metabolized to a hydroxylated product which has been tentatively assigned the structure 3-hydroxycotinine[3] (**3**). McKennis, *et al.*, have reported the isolation of hydroxycotinine from smoker's urine[3] and also from the urine of dogs,[4] rats,[5] and humans[6] treated with cotinine. Others have observed this metabolite in tissue incubates of nicotine and cotinine.[7] Elemental analysis and ir data suggested the presence of a hydroxylactam system. Conversion of the metabolite to optically active cotinine of known absolute configuration established that the asymmetric center at C-2′ of nicotine is unaltered and suggested that the newly introduced OH function is located either at C-3 or C-4 of the pyrrolidinone ring. The preparation of metabolic hydroxycotinine as a minor product obtained from the diazotization of a 3-aminocotinine led McKennis to propose 3-hydroxycotinine as the structure of the metabolite.[3] However, because of the ease with which aliphatic diazo compounds undergo rearrangement,[8] the authors noted that this assignment must be considered tentative. No attempt was made to establish the configuration of the metabolite at C-3.

As part of our studies on the mechanisms of oxidative metabolism of N-containing compounds,[9] we have undertaken an analysis of the metabolism of cotinine. In order to obtain an authentic sample of metabolic hydroxycotinine, (*S*)-cotinine (**2**), prepared by oxidation of (*S*)-nicotine,[10] was administered iv to a 4-kg male rhesus monkey and the organic soluble base fraction isolated from the 48-hr urine. The material corresponding to hydroxycotinine was purified by preparative tlc or alumina column chromatography

†Presented in part to the Medicinal Chemistry Division, 162nd National Meeting of the American Chemical Society, Sept 1971, Washington, D. C. Support from Program Project Grant GM 16496 and University of California Academic Senate Grant ASC-31 are gratefully acknowledged.

‡Predoctoral fellow supported by Patent Board funds of the University of California.