Defensive substances of *Coccinella transversoguttata* and *Hippodamia caseyi*, ladybugs indigenous to western Canada

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The defensive substances of two species of ladybug indigenous to western Canada have been isolated. The known compounds precoccinelline (**1**) and coccinelline (**2**) are present in *Coccinella transversoguttata*, and hippodamine (**3**) and convergine (**4**) in *Hippodamia caseyi*. From the latter insect we have also isolated two new alkaloids, a new base, *N*-oxide pair, which we have named hippocasine and hippocasine oxide. The structure of hippocasine oxide (**8**) was established by spectroscopic methods and confirmed by X-ray analysis of the hydrochloride (**9***d* and Fig. 1).

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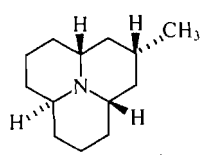
On a isolé les substances défensives de deux espèces de coccinelles indigènes à l'ouest canadien. Les composés connus, précoccinelline (**1**) et coccinelline (**2**), sont présents dans la *Coccinella transversoguttata* et l'hippodamine (**3**) et la convergine (**4**) dans *l'Hippodamia caseyi*. On a isolé deux nouveaux alcaloïdes à partir de ce dernier insecte; il s'agit d'une nouvelle paire de base et d'oxyde d'azote correspondant que l'on a nommés respectivement hippocasine et oxyde d'hippocasine. On a établi la structure de l'oxyde d'hippocasine (**8**) par des méthodes spectroscopiques et on l'a confirmée par analyse de rayon-X du chlorhydrate (**9***d* et fig. 1).

[Traduit par le journal]

The polka-dotted beetles (ladybugs) because of their voracious appetite for aphids, mealy bugs, and scale insects have long been known as a friend of man (1). These colorful beetles, members of the family Coccinellidae, not only have potential economic impact in the biological control of plant pests on agricultural crops but also exhibit an interesting protective mechanism against their predators through secretion of defensive compounds associated with 'reflex bleeding' (2).

In recent years, Tursch and co-workers have investigated the defensive substances present in several species of Coccinellidae. They found that the defensive compounds are bitter-tasting alkaloids, many of which are related to 2-methylperhydro-9b-azaphenalene and the corresponding *N*-oxides. These include precoccinelline (**1**), coccinelline (**2**) (3), hippodamine (**3**), convergine (**4**) (4), myrrhine (**5**) (5), and propyleine (**6**) (6).



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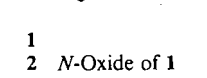
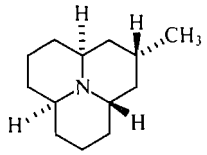


2 *N*-Oxide of **1**



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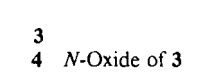
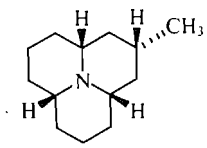


4 *N*-Oxide of **3**



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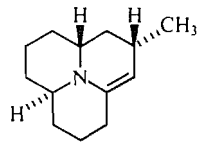

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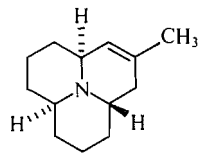

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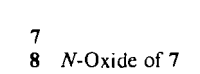


8 *N*-Oxide of **7**

We have examined the defensive substances present in two species of ladybugs indigenous to western Canada: *Coccinella transversoguttata* Faldermann and *Hippodamia caseyi* Johnson. At this time we wish to report the isolation and characterization of precoccinelline (**1**) and coccinelline (**2**) from *C. transversoguttata*;

TABLE 1. Alkaloids isolated from *H. caseyi*

Compound	R_f	M^+	Empirical formula
C	0.72	191	$C_{13}H_{20}N^*$ ($M^+ - 1$)
D	0.65	193	$C_{13}H_{23}N$
E	0.38	209	$C_{13}H_{23}NO^*$
F	0.31	207	$C_{13}H_{21}NO^*$

*Determined by high resolution mass measurement.

hippodamine (**3**), convergine (**4**), and two new alkaloids, hippocasine (**7**) and hippocasine oxide (**8**), from *H. caseyi*.

The beetles (~500 *Coccinella transversoguttata*) were blended with methanol and the supernatant was separated and partitioned between methanol–water (9:1) and pentane. Concentration of the aqueous methanol extract followed by chromatographic separation (alumina) led to the isolation of two alkaloids: compound *A* ($C_{13}H_{23}N$) and compound *B* ($C_{13}H_{23}NO$). That *B* was the *N*-oxide of *A* was shown as follows. Treatment of compound *A* with *m*-chloroperbenzoic acid followed by hydrochloric acid gave compound *B* hydrochloride (superimposable ir, co-tlc). Compound *B*, which crystallizes from acetone, shows a mass spectral fragmentation pattern similar to that reported for the 2-methyl-9b-azaphenalene *N*-oxides (3). Its nmr spectrum displays a methyl doublet (δ 1.03) and a three-proton low-field multiplet (δ 3.38). Its ^{13}C nmr spectrum shows eight signals. The evidence presented is consistent with that reported for coccinelline (3). Comparison of the spectra of compound *B* with that of an authentic sample of coccinelline revealed the two were identical. Thus compound *B* is coccinelline, **2**, and compound *A* is precoccinelline, **1**.

The extract from *Hippodamia caseyi* Johnson was obtained by the method described above. Chromatographic separation (alumina) of the crude extract led to the isolation of four alkaloids. The mass spectra of these compounds suggested the presence of two free base *N*-oxide pairs (see Table 1).

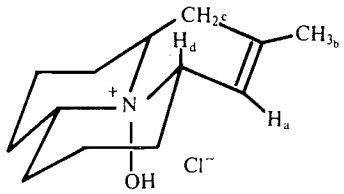
The relationship between compounds *C* and *F* and compounds *D* and *E* was shown in the following way. Treatment of compound *D* with *m*-chloroperbenzoic acid (8) followed by salt formation gave compound *E* hydrochloride (co-tlc, superimposable ir) while treatment of compound *C* with methanolic hydrogen peroxide (9), then salt formation, gave compound *F* hydrochloride (co-tlc, superimposable ir). As well, pyrolysis of compound *E* (150–155 °C) gave compound *D*, whereas pyrolysis of compound *F* gave compound *C*. Since the free bases, *C* and *D*, were isolated in small quantities and decomposed readily, they were characterized as their *N*-oxides.

Compound *E* ($C_{13}H_{23}NO$) was isolated as an oil. It was transformed to a crystalline hydrochloride by treatment with hydrochloric acid in acetone. The nmr spectrum shows a methyl doublet at δ 0.87, $J = 4$ Hz and low-field multiplets at δ 3.9 (1H) and δ 4.5 (2H). The mass spectrum shows a fragmentation pattern similar to that observed for the 2-methylperhydro-9b-azaphenalene *N*-oxide skeleton (3).

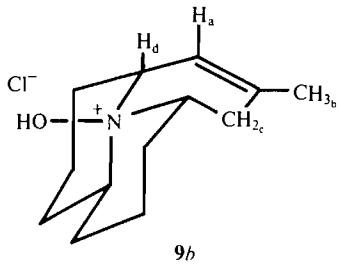
Comparison of the spectral data of compound *E* hydrochloride with that of a synthetic sample (10) of convergine hydrochloride, **4**, showed that they were identical in all respects. In addition, the ir spectrum of compound *E* hydrochloride was superimposable on that of convergine hydrochloride, **4**, isolated from *Hippodamia convergens* by Tursch and co-workers (4).

As mentioned previously compound *D* was transformed to compound *E* by treatment with *m*-chloroperbenzoic acid. Since compound *E* has been shown to be convergine, **4**, compound *D* must be hippodamine, **3**.

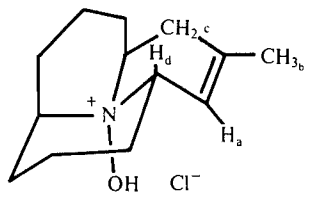
Compound *F* ($C_{13}H_{21}NO$, and for which we propose the name hippocasine oxide) is an optically active alkaloid which was isolated as an oil. It was characterized as its hydrochloride which decomposes without melting above 220 °C. The ir spectrum of hippocasine oxide hydrochloride shows salt (O—H) bands (2700–2500 cm^{-1}) and olefinic absorption (1675 cm^{-1}). The nmr spectrum shows a vinyl methyl (δ 1.78), a low-field, three-proton multiplet (δ 4.03–4.27, —CH—N—) and an olefinic proton (δ 5.42). Long range coupling between the olefinic proton and the vinyl methyl group as well as with an allylic methylene was established by double irradiation experiments. The mass spectral fragmentation pattern of hippocasine oxide hydrochloride is similar to that observed for the 2-methylperhydro-9b-azaphenalene *N*-oxide system except that each major fragment is two mass units less. Assuming the same carbon skeleton, this data is consistent with the structures **9***a*, **9***b*, **9***c*, **9***d*.



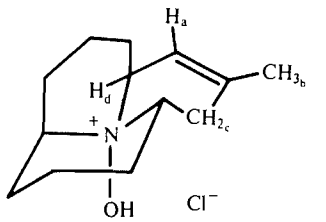
9*a*



9*b*



9*c*



9*d*

Further analysis of the nmr spectrum of hippocasine oxide hydrochloride shows that the olefinic proton H_a, is a nine-line multiplet resulting from the overlapping of a doublet of sextets with coupling constants $J_{ab} = J_{ac} = 1.5$ Hz, $J_{ad} = 4.5$ Hz. Examination of molecular models of compounds, **9***a*, **9***b*, **9***c*, and **9***d* reveals that only in **9***d* is the dihedral angle between H_a and H_d small enough to account for the observed coupling (11). In the other three isomers (**9***a*, **9***b*, **9***c*) $\theta_{ad} \sim 90°$ and thus J_{ad} would be expected to be much smaller (11).

In order to verify that hippocasine oxide is **9***d* ≡ **8**, transformation of **8** to convergine **4**, was attempted. Catalytic hydrogenation of **8** over palladium–charcoal led to the formation of a base $C_{13}H_{23}N$ which was transformed to the *N*-oxide hydrochloride in the usual manner. Comparison of the ir spectrum of this compound with that of convergine hydrochloride showed that the two compounds were not identical. Catalytic hydrogenation of hippocasine oxide may proceed from the α-face to give the compound with an axial methyl group. Attempted dissolving metal reduction of **8** was unsuccessful.

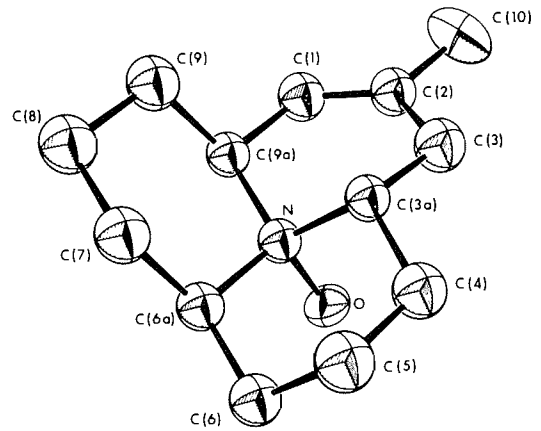


FIG. 1. The molecular structure of hippocasine oxide hydrochloride.

Since small quantities of hippocasine oxide remained, its structure was confirmed by X-ray analysis of the hydrochloride. The result is shown in Fig. 1. The average C—C bond length and the C=C distance are normal. The average C—N distance of 1.541 Å agrees well with that of 1.548 Å found in convergine hydrochloride (4). The N—O bond length of 1.430 Å is similar to that found in convergine hydrochloride and coccinelline hemihydrochloride (3*b*). Bond lengths and intramolecular angles are

TABLE 3. Interatomic distances with estimated standard deviations in parentheses

Bond	Distance (Å)	Bond	Distance (Å)
N—C9a	1.523(8)	C6—C6a	1.515(9)
N—C6a	1.541(8)	C7—C8	1.52(1)
N—C3a	1.560(8)	C8—C9	1.505(9)
N—O	1.430(7)	C9—C9a	1.511(9)
C3a—C4	1.504(9)	C9a—C1	1.488(9)
C3a—C3	1.514(9)	C1—C2	1.32(1)
C4—C5	1.50(1)	C2—C3	1.50(1)
C5—C6	1.56(1)	C2—C10	1.52(1)

TABLE 4. Intramolecular angles with estimated standard deviations in parentheses

Bonds	Angle (deg)	Bonds	Angle (deg)
O—N—C3a	105.3(4)	C7—C6a—N	111.3(6)
O—N—C6a	107.6(5)	C6a—C7—C8	109.8(6)
O—N—C9a	109.6(5)	C7—C8—C9	109.8(6)
C3a—N—C6a	112.3(5)	C8—C9—C9a	113.5(6)
C3a—N—C9a	110.8(5)	C9—C9a—C1	112.4(6)
C6a—N—C9a	111.0(5)	C9—C9a—N	112.5(6)
N—C3a—C4	109.6(6)	C1—C9a—N	110.2(5)
N—C3a—C3	108.9(6)	C9a—C1—C2	124.4(7)
C4—C3a—C3	108.9(6)	C1—C2—C10	122.8(8)
C3a—C4—C5	114.9(6)	C1—C2—C3	121.9(7)
C5—C6—C6a	111.0(6)	C10—C2—C3	115.4(7)
C6—C6a—C7	114.3(6)	C2—C3—C3a	116.1(6)
C6—C6a—N	110.3(5)		

TABLE 5. Atomic parameters

(*a*) Atom coordinates

Atom	*x*	*y*	*z*	*B*
C1	0.6952(2)	0.2500	0.3116(3)	4.41
O	0.1710(5)	0.0605(6)	0.5808(6)	3.41
C10	−0.052(1)	0.256(2)	0.926(1)	5.86
N	0.2603(5)	0.1930(7)	0.6525(6)	2.74
C1	0.1864(7)	0.177(1)	0.9144(9)	3.56
C2	0.0686(6)	0.251(1)	0.8461(8)	3.40
C3	0.0462(7)	0.336(1)	0.6809(9)	3.93
C3a	0.1711(7)	0.3525(9)	0.6095(9)	2.85
C4	0.1323(7)	0.377(1)	0.4241(9)	3.88
C5	0.2510(8)	0.378(1)	0.3440(9)	4.41
C6	0.3355(7)	0.216(1)	0.3870(8)	3.52
C6a	0.3838(7)	0.1928(9)	0.5731(8)	3.13
C7	0.4931(7)	0.3141(9)	0.6586(9)	3.61
C8	0.5410(7)	0.285(1)	0.8435(9)	4.09
C9	0.4193(7)	0.287(1)	0.9199(8)	3.63
C9a	0.3063(7)	0.1692(9)	0.8388(8)	2.89

(*b*) Anisotropic parameters

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C8	0.061(1)	0.0201(8)	0.080(1)	−0.003(1)	0.007(1)	0.002(1)
O	0.040(3)	0.019(3)	0.064(4)	−0.001(3)	0.001(3)	−0.010(3)
C10	0.079(7)	0.058(6)	0.087(7)	0.009(8)	0.044(6)	−0.006(8)

given in Tables 3 and 4 and atomic parameters are given in Table 5. Structure factor tables are available (Table 2).[1]

As discussed earlier compound *C* was transformed to compound *F* by treatment with methanolic hydrogen peroxide. Since compound *F*, hippocasine oxide, has been shown to be **8**, it follows that compound *C*, hippocasine, is represented by structure **7**.

[1]Table 2 is available, at a nominal charge, from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Canada K1A 0S2.

Experimental

Optical rotatory dispersion (ord) spectra were measured in methanol using a Jasco Optical Rotatory Dispersion Recorder Model ORV/UV-5.

Infrared spectra (ir) were measured in KBr unless otherwise specified using a Perkin-Elmer 421 grating ir spectrophotometer or a Unicam SP 1000 grating ir spectrophotometer.

Proton magnetic resonance spectra were measured in $CDCl_3$ unless otherwise specified using a Varian Associates HA 100 spectrophotometer with TMS as internal standard. ^{13}C magnetic resonance spectra were measured in $CDCl_3$ using a Varian HA 100 15 in. system interfaced to a Digilab FTS/NMR 3 data system and pulse unit.

Mass spectra were recorded on an A.E.I. Model MS-9 mass spectrophotometer and are reported as *m/e* (relative intensity). Unless diagnostically significant, only peaks at least 20% as intense as the base peak are reported. For the hydrochloride of the *N*-oxides, the sample was allowed to stabilize at 155 °C, then recorded.

Optical density measurements were recorded in methanol using a Perkin-Elmer 141 Polarimeter.

Melting points were recorded on a hot-stage Fisher-Johns melting point apparatus and are uncorrected.

Insect Material

Coccinella transversoguttata were collected in the Edmonton area and identified by J. Belicek, Department of Entomology, University of Alberta, Edmonton, Alberta.

Hippodamia caseyi Johnson were collected from their over-winter site (7) in south-central British Columbia and identified by R. D. McMullen, Entomologist, Canada Department of Agriculture, Research Station, Summerland, British Columbia.

Extraction and Isolation of the Alkaloids of Coccinella transversoguttata

Approximately 500 ladybugs (*C. transversoguttata*) were extracted with methanol in a Waring blender. The extract was filtered, diluted with water (10% by volume), then washed several times with pentane. The aqueous methanol extract was concentrated *in vacuo* to give a crude extract. Thin layer chromatography (alumina, chloroform) of this material revealed the presence of two basic components as well as nonbasic material.

Elution chromatography of the extract (chloroform) over alumina led to fractions rich in compound *A* (150 mg). Further purification by acid–base extraction gave 90 mg compound *A*. Elution with chloroform–methanol (100:1) gave compound *B* (27 mg).

Precoccinelline, 1

Compound *A* was isolated as an oil: nmr δ 0.97 (d, 3H, J = 5 Hz, C*H*$_3$), 3.0 (m, 3H, N—C*H*); *Exact Mass* calcd. for $C_{13}H_{23}N$: 193.1830; found (ms): 193.1821; ms *m/e* 193.1821 (58), 192 (100), 178 (21), 164 (23), 151 (49), 150 (46), 137 (35), 136 (20), 41 (22).

Conversion of Precoccinelline, 1, to Coccinelline, 2

Compound *A* (40 mg) and *m*-chloroperbenzoic acid (40 mg) in chloroform were allowed to stand in the refrigerator overnight. The mixture was concentrated and the product isolated by preparative thin layer chromatography over alumina (chloroform–methanol, 20:1). Compound *A N*-oxide was converted to its hydrochloride salt in the usual manner. The ir spectrum of compound *A N*-oxide hydrochloride was superimposable on that of compound *B* hydrochloride.

Coccinelline, 2

Coccinelline was isolated as a solid which was recrystallized from acetone: mp >215 °C (chars); ir 3300, 2950, 2910, 2860, 2720, 2650, 1450, 1440, 1430, 1370, 1350, 1340, 1320, 1290, 1280, 1260, 1195, 1180, 1160, 1120, 1100, 1040, 990, 970, 940, 900, 870, 850, 840, 820, 800, 650, 600 cm^{-1}; nmr δ 1.03 (d, 3H, J = 5 Hz, C—C*H*$_3$), 3.38 (m, 3H, N—C*H*); ^{13}C nmr δ ($CDCl_3$), 73.9, 58.7, 36.0, 30.7, 27.6, 25.7, 21.5, 18.3; *Exact Mass* calcd. for $C_{13}H_{23}NO$: 209.1780; found (ms): 209.1785; ms *m/e* 209.1785 (9), 129 (100), 191 (64), 190 (75), 178 (22), 176 (95), 164 (30), 151 (49), 150 (62), 137 (41), 136 (41), 134 (20), 122 (24), 82 (23), 69 (23), 68 (23), 67 (30), 60 (22), 55 (67), 54 (26), 53 (26), 43 (57), 41 (100).

Coccinelline was converted to its hydrochloride salt in the usual way and recrystallized from methanol–ether: mp 220 °C (dec. without melting); ir (KBr) 3300, 2940, 2920, 2870, 2850, 2500, 1520, 1490, 1460, 1445, 1430, 1380, 1370, 1300, 1280, 1260, 1210, 1180, 1170, 1120, 1100, 1030, 990, 985, 970, 950, 910, 900, 880, 855, 770, 610 cm^{-1}.

Extraction and Isolation of the Alkaloids of Hippodamia caseyi Johnson

Approximately 5000 ladybugs (*H. caseyi* Johnson) were extracted with methanol in a Waring blender. The extract was filtered, diluted with water (10% by volume), then washed several times with pentane. The aqueous methanol extract (1.5 l) was stored in the refrigerator; further isolation work was carried out on portions of this extract.

In one experiment, the aqueous methanol extract (500 ml) was concentrated *in vacuo* to give an oil (2.9 g). Thin layer chromatography of this material (alumina, chloroform–methanol 50:1) showed the presence of four basic (Dragendorf positive) components (R_f: *C* 0.72, *D* 0.65, *E* 0.38, *F* 0.31) as well as nonbasic material.

Elution chromatography over alumina (BDH, 300 g) with chloroform led to isolation of fractions rich in base *C* (30 mg) and base *D* (47 mg). Each of these fractions was purified further by acid–base extraction to give base *C* (10 mg) and base *D* (12 mg). Elution with chloroform–methanol (50:1) led to isolation of base *E* (17 mg) and base *F* (47 mg).

The free bases appeared to decompose on standing and thus were converted to and stored as their hydrochloride salts.

General Method of Preparation of Hydrochlorides

The alkaloid was dissolved in acetone and a few drops of concentrated hydrochloric acid were added. The mixture was allowed to stand at room temperature for about 5 min, then concentrated *in vacuo*. If the residue was discolored it was dissolved in methanol and filtered through a small plug of charcoal (AU-4).

Hippodamine, 3

Compound *D* (R_f 0.65) was isolated as an oil: ir (neat) 2920, 2850, 1450 (sh), 1440, 1375, 1350, 1340, 1320, 1280, 1220, 1190, 1155 (sh), 1145, 1130, 1115, 1100, 1070, 1020, 885, 835, 830, 800, 710 cm^{-1}; ms *m/e* 193 (55), 192 (100), 178 (34), 165 (44), 152 (20), 151 (83), 150 (65), 149 (20), 137 (38), 136 (31), 111 (23), 109 (28), 97 (38), 96 (24), 95 (34), 85 (38), 83 (59), 81 (38), 71 (38), 68 (51), 66 (34), 57 (62), 55 (80), 43 (68), 41 (68); nmr δ 0.86, (d, J = 6 Hz), 1.5 (m), 1.9 (m), 2.2 (m), 3.0 (m).

Conversion of Hippodamine, 3, to Convergine Hydrochloride, 4

Compound *D* (12 mg) and *m*-chloroperbenzoic acid (15 mg) in chloroform were allowed to stand in the

refrigerator overnight. The reaction mixture was concentrated and the residue chromatographed over alumina (BDH). Elution with chloroform gave compound *D N*-oxide (6 mg).

Compound *D N*-oxide was converted to a hydrochloride in the usual manner. The ir spectrum of compound *D N*-oxide hydrochloride was superimposable on that of compound *E* hydrochloride.

Convergine, 4

Compound *E*, R_f 0.38, was isolated as an oil. It was converted to its *hydrochloride*; chars without melting >215 °C: ir 2940, 2880, 2550 (br), 1500, 1450, 1380, 1360, 1330, 1315, 1305, 1265, 1210, 1205, 1160, 1095, 1020, 1010, 1000, 970, 940, 930, 900, 890, 850, 820, 810, 740, 610 cm^{-1}; *Exact Mass* calcd. for $C_{13}H_{23}NO$: 209.1780; found (ms): 209.1794; ms *m/e* 209.1794 (20), 193 (34), 192 (100), 191 (24), 178 (20), 176 (36), 164 (28), 151 (32), 150 (32), 55 (22), 41 (28); nmr δ 0.87, (3H, d, J = 4 Hz), 3.9 (1H, m), 4.5 (2H, m).

The spectral data for compound *E* hydrochloride is identical with that obtained for a synthetic sample of convergine hydrochloride prepared in these laboratories (10). Additionally, the ir spectrum of the naturally occurring convergine hydrochloride is superimposable on the ir spectrum of convergine hydrochloride isolated by Tursch and co-workers (4).

Conversion of Convergine, 4, to Hippodamine, 3

Convergine (2 mg) was heated in a glass tube in a sublimation block at 150–155 °C for about 1 h. The portion of the glass tubing outside the block was cooled by a wick dipped in ice water. The oil obtained from this distillation has the same R_f (0.65) and ir spectrum as hippodamine, **3**.

Hippocasine, 7

Compound *C*, R_f 0.72 was isolated as an oil: ir (neat) 2880, 2820, 1625 (>C=C<), 1490, 1480, 1445, 1430, 1370, 1330, 1210, 1190, 1105, 1070, 1045, 1020, 955, 910, 880, 830, 820, 800, 735, 710 cm^{-1}; *Exact Mass* calcd. for $C_{13}H_{20}N$: 190.1596; found (ms): 190.1592; ms *m/e* 191 (60), 190.1592 (100), 176 (84), 162 (49), 148 (38), 147 (37), 120 (25), 107 (22), 94 (26), 93 (25), 91 (25), 81 (26), 70 (28), 66 (29), 55 (42), 43 (35), 41 (65).

Conversion of Hippocasine, 7, to Hippocasine Oxide, 8

Compound *C* (14 mg) and 30% hydrogen peroxide (1 drop) in methanol (2 ml) were allowed to stand at room temperature. Additional hydrogen peroxide (1 drop) was added after 2 h and 6 h. After 48 h, platinum black (few grains) was added. When oxygen evolution was complete the reaction mixture was filtered and concentrated. The residue was converted to the hydrochloride to give compound *C N*-oxide hydrochloride (13 mg). The ir spectrum of this compound was identical in all respects with that of hippocasine oxide hydrochloride.

Hippocasine Oxide, 8

Compound *F*, R_f 0.31, was isolated as an oil. It was converted to a *hydrochloride* which was crystallized from ethyl acetate containing a trace of methanol (dec. without melting above 220 °C), $[\alpha]_D^{25}$ +14.8 (*c*, 0.73, methanol): ir 2940, 2900, 2860, 2700, 2500, 1675, 1500, 1460, 1425, 1380, 1360, 1350, 1335, 1310, 1290, 1280, 1260, 1230, 1200, 1150, 1140, 1110, 1090, 1065, 1010, 970, 955, 925, 895, 870, 845, 835, 825, 800, 790, 730, 690, 650, 640, 620 cm^{-1}; *Exact Mass* calcd. for $C_{13}H_{21}NO$: 207.1623; found (ms): 207.1630; ms *m/e* 207.1630 (20), 191 (42), 190 (100), 188 (94), 176 (58), 174 (36), 162 (37), 160 (27), 148 (26), 94 (20) 55 (30), 43 (22), 41 (45); nmr (CD_3OD) δ 1.78, (br s, 3H, C=C—CH_3), 1.8 (m), 2.25 (m), 4.0–4.3, (m, 3H, —CH—N—); 5.42 (nine line m, 1H, J = 1.5, 4.5 Hz, C=C—H); irradiation at 5.42 sharpens 1.78 and 2.25; ord $[\alpha]_{400}$ +15.8, $[\alpha]_{300}$ +42.1, $[\alpha]_{250}$ +94.7 (*c* 1.9, methanol).

Conversion of Hippocasine Oxide, 8, to Hippocasine, 7

Hippocasine oxide (2 mg) was pyrolyzed as described for convergine. The oil obtained in the distillate had identical tlc behavior (R_f 0.72) and superimposable ir spectra with that of hippocasine, **7**

Catalytic Hydrogenation of Hippocasine Oxide

A solution of hippocasine oxide hydrochloride (14 mg) in methanol containing palladium–charcoal (~10 mg) was hydrogenated under 48 psi hydrogen overnight. The solution was filtered and concentrated to give an oil (7 mg): *m/e* M^+ 193, 192 (base), 178, 164, 151, 150.

The hydrogenation product was dissolved in chloroform and treated with *m*-chloroperbenzoic acid (10 mg) for 12 h. The reaction mixture was concentrated and chromatographed over alumina (BDH). The *N*-oxide (5 mg) thus obtained was converted to a hydrochloride salt in the usual way: ir 3500, 2930, 2920, 2700 (br), 1600 (br, hydrate), 1450, 1430, 1390, 1355, 1340, 1310, 1270, 1220, 1160, 1130, 1100, 1065, 1030, 1020, 1000, 975, 940, 900, 870, 860, 830, 820, 810, 750, 670 cm^{-1}.

Crystal Structure Determination of Hippocasine Oxide Hydrochloride

Crystal data:

$C_{13}H_{22}NOCl$ f.w. = 243.5

Monoclinic, a = 10.011(2), b = 8.117(2), c = 8.312(2), β = 104.02(2), V = 655.3, ρ_o = 1.24(1), Z = 2, ρ_c = 1.234 (20 °C; CuK_α, λ = 1.54182 Å).

The molecule crystallizes in the space group $P2_1$. A total of 766 significantly above background reflections were measured on a Picker FACS I automatic diffractometer, using nickel filtered CuK_α radiation. The data were corrected for Lorentz and polarization effects and structure factor amplitudes and standard deviations calculated, using an uncertainty factor of 0.03 (12). Absorption and extinction corrections were not applied to the data.

The structure was solved by conventional Patterson and Fourier techniques and refined to a value for R = 0.057. In the refinement, each structure amplitude was given a weight which was the inverse of its standard deviation and the quantity minimized was $\Sigma\omega(|F_o| - |F_c|)$. The chlorine, oxygen, and methyl carbon atoms were refined anisotropically, all other atoms having isotropic temperature factors. The hydrogen atoms of the tricyclic system were included in calculated positions, with temperature factors 10% greater than the atom to which they were attached, and were not refined. The methyl hydrogens were located in a difference electron density map, and included in the refinement as a hindered rotor (13). No feature in the difference electron density map could be attributed to the hydrogen of the hydrochloride system and this atom was omitted from the calculations.

Scattering factors for all heavy atoms were those of Cromer (14), while for hydrogen those of Stewart, Davison, and Simpson were used (15). Anomalous dispersion factors were included for chlorine.

The statistically favoured configuration (16) is that shown in Fig. 1, but there was very little difference between the weighted R values for the two hands, and an unambiguous choice could not be made.

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