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BENZOPHENANTHRIDINE ALKALOIDS FROM THE STEM BARK OF A *ZANTHOXYLUM* SPECIES

KWOK MING NG, ALEXANDER I. GRAY and PETER G. WATERMAN

Phytochemistry Research Laboratories, Department of Pharmacy, Pharmaceutical Chemistry, University of Strathclyde, Glasgow G1 1XW, Scotland, U.K.

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Key Word Index—*Zanthoxylum spinosum*; *Z. coriaceum*; Rutaceae; alkaloids; benzophenanthridines; dihydrobenzophenanthridines; chemical taxonomy.

Abstract—From the stem bark of a *Zanthoxylum* species collected on Grand Cayman Island 12 benzophenanthridine alkaloids have been isolated of which five are new. The novel alkaloids have been identified on the basis of spectral analysis as decarine acetate, 6-carboxymethyldihydrochelerythrine, 6-(4-methyl-2-oxopentanyl)dihydrochelerythrine; chelelactam, 6-[3'-(2-oxopyrrolidinyl)]-dihydrochelerythrine and caymandimerine, 2,2-di[6'-(dihydrochelerythryl)]-acetaldehyde.

INTRODUCTION

It is stated that *Zanthoxylum spinosum* (L.) Sw. (Rutaceae) is a shrub or small tree common in arid exposed limestone areas on a number of islands in the Caribbean, including Grand Cayman [1]. However, in the recent 'Flora of the Cayman Islands' Procter [2] recognised only two species of *Zanthoxylum*; *Z. flavum* Vahl. and *Z. coriaceum* A. Rich. and indicated that *Z. spinosum* was very difficult to differentiate from the latter and that material from Grand Cayman should be referred to *Z. coriaceum*. Previous studies on *Z. coriaceum* collected from the mainland of Central America have revealed the presence of quaternary aporphines, benzophenanthridines, amides and amines [3].

In this paper we report the results of an examination of a stem bark sample of a *Zanthoxylum* species collected in Grand Cayman and identical to material identified as *Z. spinosum* (as described by Adams [1]) in the Herbarium of the Mosquito Research and Control Unit on the island.

RESULTS AND DISCUSSION

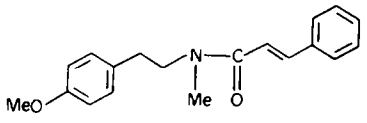
The ground stem bark was extracted sequentially with petrol (bp 60–80°), ethyl acetate and methanol. On concentration the petrol extract gave a precipitate of the amide herclavine (**1**). The extract was then subjected to preparative centrifugal TLC which yielded small amounts of two common benzophenanthridines, norchelerythrine (**2**) and dihydrochelerythrine (**3**).

Concentration of the ethyl acetate extract followed by column chromatography over silica gel and then preparative TLC yielded eight alkaloids including further **2** and **3**, decarine (**4**), oxychelerythrine (**5**) and 6-acetonyldihydrochelerythrine (**6**). Another was characterised as decarine acetate (**7**) which appears to be a novel natural product.

One of the remaining two alkaloids analysed for $C_{23}H_{21}NO_6$ and showed the typical TLC characteristics of a dihydrobenzophenanthridine (blue fluorescene, compound turns yellow on prolonged exposure to air or light). The IR spectrum revealed the presence of both hydroxyl (3450 cm^{-1}) and carbonyl (1700 cm^{-1}) functions. The ^1H NMR spectrum showed typical aromatic protons for a benzophenanthridine with the chelerythrine substitution pattern confirming that the compound was a dihydrochelerythrine substituted at C-6 (substituent $C_2H_3O_2$). The remaining feature of the NMR spectrum was a 1 H triplet at δ4.89 and a 2H doublet at δ2.40 (J = 7.7 Hz) which can be assigned to a $CH–CH_2$ system. On methylation a mono-methyl ester was formed indicating the presence of a carboxylic acid group. On the basis of this evidence the alkaloid was identified as 6-carboxymethyldihydrochelerythrine (**8**). This alkaloid has previously been reported to occur in *Zanthoxylum simulans* Hance [4] but no data have been published.

The remaining alkaloid from the ethyl acetate extract also gave reactions similar to dihydrochelerythrine. The EIMS revealed the highest m/z fragment as 391 and the expected base peak for m/z 348. The ^1H NMR showed six aromatic protons in the chelerythrine substitution pattern. A doublet at δ5.06 could be assigned to H-6 and spin decoupling revealed this to be linked with a doublet of triplets at δ2.02 which was in turn linked to a doublet at 8.78 for an aldehydic proton (1710 cm^{-1}). These last two signals integrated for half that of the H-6 resonance and the multiplicity of the δ2.02 resonance required it to be interacting with two protons. This would not be possible for a simple 6-substituted dihydrochelerythrine and requires that the compound be a symmetrical dimer in which two benzophenanthridine units are linked with C-2 of acetaldehyde, as in (**9**). This was substantiated by FABMS which revealed m/z 739 $[M+H]^+$ (empirical formula $C_{44}H_{38}N_2O_9$, 738). All previous benzophenanthridine dimers [5] have an acetonyl linking group rather than the acetaldehyde found here. The dimer has been given the trivial name caymandimerine.

After concentration the methanol extract was partitioned between ethyl acetate and water. Column chromatography of the ethyl acetate soluble portion yielded **2**, **4**, **5**, **6** and **8** followed by four further alkaloids two of which were identified as the commonly encountered chelerythrine (**10**) and bocconoline (**11**).



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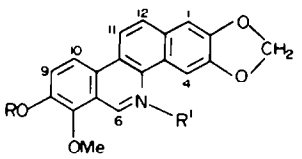
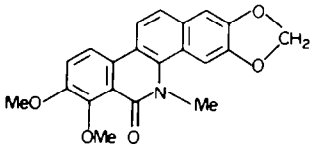


	R	R'
2	Me	–
4	H	–
7	Ac	–
10	Me	$^{\oplus}$Me



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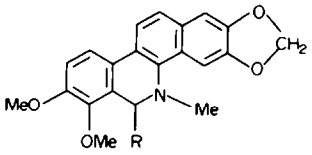
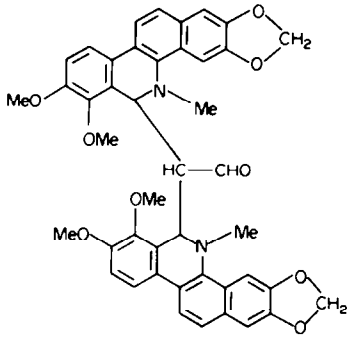


	R
3	H
6	CH_2COMe
8	CH_2COOH
11	CH_2OH
12	$CH_2COCH_2CH(Me)_2$



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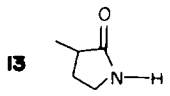

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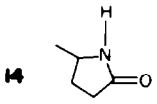

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The major new alkaloid analysed for $C_{27}H_{29}NO_5$ and once more had the characteristics of a 6-substituted dihydrochelerythrine type. The EIMS showed a significant fragment at m/z 404 $[M - C_3H_7]^+$ due to loss of part of the C-6 substituent ($C_6H_{11}O$). The ^{1}H NMR spectrum revealed an ABX system attributable to H-6 and an adjacent methylene group. These were separated from a second series of signals that could be assigned to a CH_2–$CH(Me)_2$ group. Linking these two systems through a carbonyl (1715 cm^{-1}) requires a 4-methylpentan-2-one moiety and leads to the assignment of structure **12** for this alkaloid. The ^{13}C NMR spectrum complied with the proposed structure.

The final alkaloid again showed the characteristics of a dihydrochelerythrine type. Accurate mass measurement revealed m/z 432 ($C_{25}H_{24}N_2O_5$) for the molecular ion with loss of C_4H_6NO to give the typical m/z 348 base peak. The nature of the C-6 substituent was established primarily from the ^{1}H NMR spectrum which revealed a CH(C-6)–CH–CH_2–CH_2 system and an additional N–H proton. The IR spectrum, run in CCl_4 to minimise H-bonding, revealed a sharp band at 3450 cm^{-1} characteristic of a secondary lactam [6] while the normal spectrum showed a strong band at 1700 cm^{-1} for a carbonyl. These data suggested that the substituent was a 2-oxopyrrolidine linked to the dihydrobenzophenanthridine to give either **13** or **14**. The identification of the isolate as **13** was confirmed by the ^{13}C NMR spectrum which showed a triplet at 40.1 ppm for a methylene α to the NH of the pyrrolidone. If this had been α to the carbonyl (pyrrolidine substituted through C-5') it would have resonated at about 30 ppm [6]. The novel alkaloid is therefore assigned structure **13** and the trivial name of chelelactam.

The alkaloid profile of this material of *Zanthoxylum* is almost entirely one of benzophenanthridines. Quaternary aporphinium compounds may be present in the methanol extract as minor constituents but there was no trace in the stem bark extracts of the quinoline alkaloids that charac-

terize many species of *Zanthoxylum*. In this sense the profile is very similar to that found to date for *Z. coriaceum* and would not argue against Procter's view that material from Grand Cayman were assignable to that species rather than to *Z. spinosum*.

EXPERIMENTAL

Mps uncorr. UV: MeOH, IR: KBr discs. ^{1}H NMR: run at 250 MHz in $CDCl_3$ unless otherwise stated; TMS int. standard. EIMS: at 70 eV with direct probe insertion at elevated temp. Petrol refers to bp 60–80° fraction.

Plant material. A sample of stem bark was collected on the coastal bluffs at East End, Grand Cayman in August, 1981. A voucher specimen, A. I. Gray s/n, has been deposited at the Herbarium of the Royal Botanic Garden, Edinburgh.

Extraction and isolation of alkaloids. Ground stem bark (500 g) was extracted with petrol, then EtOAc, and finally MeOH. On concn the petrol extract yielded **1** (760 mg). The supernatant was then subjected to centrifugal PTLC eluting with a petrol–EtOAc gradient of increasing polarity to give **2** (14 mg) followed by **3** (6 mg). After concn the EtOAc extract was subjected to CC eluting with petrol–EtOAc mixtures to give eight alkaloid bands, some of which were further purified by centrifugal PTLC. Isolated compounds were, in order of elution, **2** (20 mg), **3** (5 mg), **4** (154 mg), **7** (11 mg), **8** (6 mg), **6** (5 mg), **5** (25 mg), **9** (4 mg). After concn to dryness the MeOH extract was partitioned between EtOAc and H_2O. Treatment of this EtOAc soluble fraction in the same manner as the EtOAc extract gave the following; **2** (10 mg), **4** (615 mg), **8** (3 mg), **6** (4 mg), **5** (6 mg), **12** (50 mg), **11** (15 mg), **13** (30 mg), **10** (20 mg).

Norchelerythrine (**2**). Found: $[M]^+$ 333.0971; $C_{20}H_{15}NO_4$ requires 333.1001. UV, IR, ^{1}H NMR, EIMS [5].

Dihydrochelerythrine (**3**). Found: $[M]^+$ 349.1272; $C_{21}H_{19}NO_4$ requires 349.1314. UV, IR, ^{1}H NMR, MS [5].

Decarine (**4**). Found: $[M]^+$ 319.0823; $C_{19}H_{13}NO_4$ requires 319.0844. UV, IR, ^{1}H NMR, EIMS [5, 7].

Oxychelerythrine (**5**). Found; $[M]^+$ 363.1093; $C_{21}H_{17}NO_5$ requires 363.1077. UV, IR, ^{1}H NMR, EIMS [5].

6-*Acetonyldihydrochelerythrine* (**6**). Found; $[M]^+$ 405.1608; $C_{24}H_{23}NO_3$ requires 405.1576. UV, IR, ^{1}H NMR, EIMS [5, 8].

Decarine acetate (**7**). Prisms from petrol–EtOAc, mp 210–212°. Found: $[M]^+$ 361.0916; $C_{21}H_{15}NO_5$ requires 361.0950. UV λ_{max} nm: 239, 266, 273, 286, 320 (+ NaOH) 259, 314, 341. IR ν_{max} cm^{-1}: 2920, 2830, 1750, 1590, 1460, 1370, 1250, 1230, 1035. ^{1}H NMR (360 MHz, $CDCl_3$): δ 9.74 (1H, *s*, H-6), 8.73 (1H, *s*, H-4), 8.37, 7.57 (2H, AB*q*, J = 9.1 Hz, H-10, H-9), 8.35, 7.86 (2H, AB*q*, J = 8.9 Hz, H-11, H-12), 7.27 (1H, *s*, H-1), 6.14 (2H, *s*, O – CH_2 – O), 4.12 (3H, *s*, 7-OMe), 2.45 (3H, *s*, OAc). EIMS *m/z* (rel. int.): 361 (69), 319 (100), 304 (59), 290 (2), 276 (31).

6-*Carboxymethyldihydrochelerythrine* (**8**). Found: M^+ 407.1369; $C_{23}H_{21}NO_6$ requires 407.1369. UV λ_{max} nm: 230, 285, 324. IR ν_{max} cm^{-1}: 3450, 2900, 2830, 1700, 1500, 1460, 1420, 1280, 1240, 1090, 1045, 860, 820. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.72, 7.55 (1H, AB*q*, J = 8.6 Hz, H-11, H-12), 7.60, 7.02 (2H, AB*q*, J = 8.8 Hz, H-10, H-9), 7.39 (1H, *s*, H-4), 7.15 (1H, *s*, H-1), 6.07 6.08 (2H, AB*q*, J = 1.1 Hz, O – CH_2 – O), 4.89 (1H, *t*, J = 7.7 Hz, H-6), 3.97, 3.95 (2 × 3H, 2 × *s*, 7-OMe and 8-OMe), 2.64 (3H, *s* NMe), 2.40 (2H, *d*, J = 7.7 Hz, 1′-CH_2). EIMS *m/z* (rel. int.): 407 (40), 348 (100), 333 (15), 318 (15), 290 (25), 260 (3), 232 (3). **8** (5 mg) was dissolved in MeOH (5 ml) and the solution acidified with conc H_2SO_4 (1 drop). After 24 hr the reaction mixture was neutralized, diluted with H_2O and partitioned into $CHCl_3$ to give 8-methyl ester (2 mg). Found: $[M]^+$ 421.1544; $C_{24}H_{23}NO_6$ requires 421.1526. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.70, 7.48 (H-11, H-12), 7.56, 6.97 (H-10, H-9), 7.53 (H-4), 7.11 (H-1), 6.05, 6.04 (O – CH_2 – O), 4.99 (1H, *dd*, J = 10.2, 4.9 Hz, H-6), 3.97, 3.93 (2 × OMe), 3.66 (3H, *s*, COOMe), 2.64 (NMe), 2.37 (1H, *dd*, J = 14.4, 4.9 Hz, H-1′), 2.32 (1H, *dd*, J = 14.4, 10.2 Hz, H-1′).

Caymandimerine (**9**). FABMS 739 $[M^+ + 1]$. UV λ_{max} nm: 228, 283, 325. IR ν_{max} cm^{-1}: 2950, 1710, 1600, 1490, 1460, 1410, 1270, 1240, 1040. ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.78 (1H, *d*, J = 6.8 Hz, CHO), 7.59, 7.41 (4H, AB*q*, J = 8.6 Hz, H-11, H-11′, H-12, H-12′), 7.55 (2H, *s*, H-4, H-4′), 7.49, 6.93 (4H, AB*q*, J = 8.7 Hz, H-10, H-10′, H-9, H-9′), 7.05 (2H, *s*, H-1, H-1′), 6.02, 6.01 (4H, AB*q*, J = 1.1 Hz, 2 × O – CH_2 – O), 5.06 (2H, *d*, J = 5.7 Hz, H-6, H-6′), 3.88, 3.74 (4 × OMe), 2.69 (2 × NMe), 2.02 (1H, *dd*, J = 6.8, 5.7 Hz, CH). EIMS *m/z* (rel. int.): 390 (6), 372 (12), 364 (6), 348 (100), 334 (29), 318 (23), 304 (16), 290 (24), 282 (43).

Chelerythrine (**10**). Found: M^+ 348.1239; $C_{21}H_{18}NO_4$ requires 348.1236. UV, IR, ^{1}H NMR, EIMS [5].

Bocconoline (**11**). Found: M^+ 379.1436; $C_{22}H_{21}NO_5$ requires 379.1420. UV, IR, EIMS [5]. ^{1}H NMR (250 MHz, $CDCl_3$): as [5] except 6.04, 6.05 (2H, AB*q*, J = 1.5 Hz, O – CH_2 – O).

6-(4-*Methyl*-2-*oxopentanyl*)*dihydrochelerythrine* (**12**). Found: M^+ 447.2045; $C_{27}H_{29}NO_5$ requires 447.2046. UV λ_{max} nm: 231, 281, 317. IR ν_{max} cm^{-1}: 2960, 1715, 1495, 1465, 1420, 1280, 1245, 1045, 950, 800. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.71, 7.48 (2H, AB*q*, J = 8.5 Hz, H-11, H-12), 7.54, 6.95 (2H, AB*q*, J = 8.6 Hz, H-10, H-9), 7.50 (1H, *s*, H-4), 7.11 (1H, *s*, H-1), 6.04 (2H, *s*, O – CH_2 – O), 5.07 (1H, *dd*, J = 11.1, 3.3 Hz, H-6), 3.96, 3.93 (2 × OMe), 2.64 (NMe), 2.55 (1H, *dd*, J = 15.2, 11.1 Hz, H-1′), 2.18 (1H, *dd*, J = 15.2, 3.3 Hz, H-1′), 2.08 (3H, *m*, H-3′, H-4′), 0.86, 0.83 (2 × 3H, 2 × *d*, J = 6.7 Hz, $CH(Me)_2$). ^{13}C NMR (62.9 MHz, $CDCl_3$): δc *s* at 208.8 (C-2′), 150.2 (C-7), 148.1, 147.6 (C-2, C-3), 145.6 (C-8), 139.5 (C-4b), 131.1, 127.4, 127.4, 125.0, 123.3; *d* at 123.8, 119.8, 118.7, 111.6, 104.3, 100.9, 54.7 (C-6), 24.1 (C-4′); *t* at 101.0 (O – CH_2 – O), 52.9 (C-1′), 46.4 (C-3′), *q* at 61.0 (7-OMe), 55.9 (8-OMe), 42.9 (N – Me), 22.6 (2 × Me). EIMS *m/z* (rel. int.): 447 (36), 404 (2), 348 (100), 333 (5), 318 (7), 290 (16), 275 (3).

Chelelactam (**13**). Found: M^+ 432.1688; $C_{25}H_{24}N_2O_5$ requires 432.1685. UV λ_{max} nm: 229, 282, 318. IR ν_{max} cm^{-1}: (CCl_4) 3450; (KBr) 3400, 2950, 1700, 1490, 1460, 1420, 1270, 1240, 1040, 940. ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.80 (1H, *s*, H-4), 7.68, 7.45 (2H, AB*q*, J = 8.4 Hz, H-11, H-12), 7.53, 6.96 (2H, AB*q*, J = 8.6 Hz, H-10, H-9), 7.08 (1H, *s*, H-1), 6.02, 6.01 (2H, AB*q*, J = 1.6 Hz, O – CH_2 – O), 5.24 (1H, *br s*, NH), 4.85 (1H, *d*, J = 6.1 Hz, H-6), 3.92, 3.90 (2 × OMe), 3.10 (1H, *dddd*, J = 9.0, 8.4, 4.2, 0.7 Hz, H-5′), 2.97 (1H, *ddd*, J = 8.4, 8.3, 7.0 Hz, H-5′), 2.70 (NMe), 2.48 (1H, *ddd*, J = 8.7, 8.7, 6.1 Hz, H-3′), 1.74 (1H, *dddd*, J = 11.6, 9.0, 8.7, 7.0 Hz, H-4′), 1.52 (1H, *dddd*, J = 11.6, 8.7, 8.3, 4.2 Hz, H-4′). ^{13}C NMR (90.56 MHz, $CDCl_3$): δ_c *s* at 178.5 (C-2′), 152.0 (C-7), 147.9, 147.7 (C-2, C-3), 146.3 (C-8), 140.4 (C-4b), 130.9, 127.4, 126.4, 125.7, 124.1; *d* at 123.5, 119.5, 118.6, 111.5, 104.1, 101.3, 57.9 (C-3′), 44.9 (C-6); *t* at 100.9 (O – CH_2 – O), 40.1 (C-5′), 25.0 (C-4′); *q* at 60.7 (7-OMe), 55.6 (8-OMe), 42.7 (N-Me). EIMS *m/z* (rel. int.): 432 (12), 348 (100), 333 (18), 318 (9), 290 (18), 275 (3).

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REFERENCES

1. Adams, C. D. (1972) *Flowering Plants of Jamaica* p. 386. University of the West Indies, Mona.
2. Procter, G. R. (1984) *Flora of the Cayman Islands* p. 582. H.M.S.O., London.

3. Mester, I. (1983) *Chemistry and Chemical Taxonomy of the Rutales* (Waterman, P. G. and Grundon, M. F. eds) p. 31. Academic Press, London.
4. O'Sullivan, J. (1983) PhD Thesis. Trinity College, Dublin.
5. Krane, B. D., Fagbule, M. O., Shamma, M. and Gozler, B. (1984) *J. Nat. Prod.* **47**, 1.
6. Williams, D. H. and Fleming, I. (1980) *Spectroscopic Methods in Organic Chemistry*, 3rd. Edn. McGraw-Hill, London.
7. Ng, K. M., But, P. P-H., Gray, A. I., Hartley, T. G., Kong, Y-C. and Waterman, P. G. (1987). *Biochem. Syst. Ecol.* **15**, 587.
8. Waterman, P. G. and Khalid, S. A. (1981) *Biochem. Syst. Ecol.* **9**, 45.