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New Alkaloids from *Cryptolepis Sanguinolenta*

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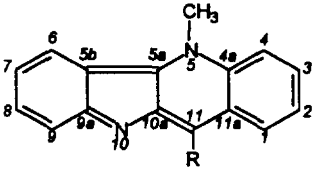
Neocryptolepine and biscryptolepine, two new alkaloids, were isolated from the root bark extract of the African medicinal plant, *Cryptolepis sanguinolenta* (Lindl.) Schlechter (Periplocaceae), and their structures elucidated on the basis of spectral evidence. The ^1H and ^{13}C NMR assignments of cryptoquindoline, a known artefact, were revised.

Seven alkaloids have so far been reported from *Cryptolepis sanguinolenta* (Lindl.) Schlechter (Periplocaceae), a plant used in traditional medicine in Central and West Africa. Until 1990, only quindoline and cryptolepine (1) had been characterised[1]. More recently, a spiro-alkaloid named cryptospirolepine[2], isocryptolepine[3] (a cryptolepine isomer), hydroxycryptolepine[1,4] (2), cryptoheptine[4], and a dimeric alkaloid cryptoquindoline (3), which was shown to be an artefact[4], were isolated and identified. This paper reports the characterisation of two new alkaloids from the same plant, *i.e.* biscryptolepine (4) and neocryptolepine (5). The ^1H and ^{13}C NMR assignments of cryptoquindoline (3), which was isolated as well, were revised.

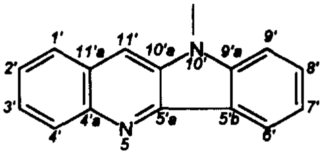
Compound 5 was obtained as an amorphous yellowish powder by column chromatography on silica gel (elution with $CHCl_3$/MeOH, increasing polarity) and preparative TLC on silica gel ($CHCl_3$/MeOH 3/1) of the organic fraction from the partition of the 80% ethanolic extract of dried root bark of *C.sanguinolenta* against $CHCl_3$. EIMS indicated the same molecular weight as cryptolepine (232), but the chromatographic behaviour was different. Similar but not identical ^1H and ^{13}C NMR spectra, compared to cryptolepine, suggested that 5 was a cryptolepine isomer. However, its NMR spectral data were different from those of isocryptolepine, a known cryptolepine isomer[3]. A detailed ^1H and ^{13}C NMR analysis performed in $CDCl_3$ at 400 MHz, including DQF-COSY, DEPT, HSQC and HMBC, led to a cryptolepine-like structure with both N atoms at the same side of the molecule (*i.e.* position 2 of the indol ring is N-substituted, whereas for cryptolepine N-substitution is at position 3). Long-range correlations observed in the HMBC spectrum are displayed in Figure 1. All ^1H and ^{13}C NMR spectral data, and all correlations observed, are in agreement with the proposed structure. Since the name isocryptolepine had already been adopted for another cryptolepine isomer[3], compound 5 was named neocryptolepine.



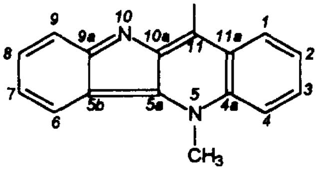
1 cryptolepine R=H

2 hydroxycryptolepine R=OH

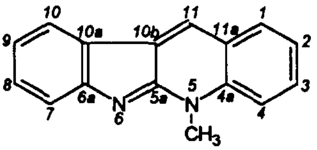
3 cryptoquindoline R=



4 biscryptolepine R=



5 neocryptolepine



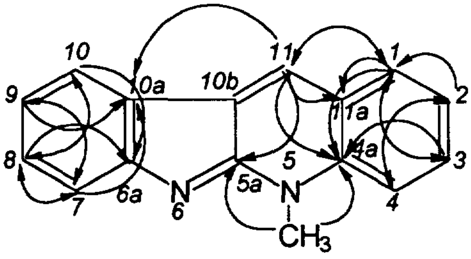


Fig.1: Long-range heteronuclear correlations observed in a HMBC experiment (optimised for $^{n}J_{CH}$=8.3 Hz) for neocryptolepine (H→C)

Neocryptolepine (5, 13 mg), $C_{16}H_{12}N_2$; Rf = 0.80 (silica gel, $CHCl_3$/MeOH 7/3); EIMS (70 eV): M/z (relative abundance) 232 [M^+] (100), 231 (91), 204 (19), 190 (11), 116 (18); UV (λ_{max}, MeOH): 432, 352, 336, 270; UV (λ_{max}, 0.01 N KOH): 433, 352, 337, 271; UV (λ_{max}, 0.01 N HCl): 348, 257, 227; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.54 (1H, s, H-11), 8.04 (1H, dd, J=7.6 Hz, 1.0 Hz, H-10), 7.99 (1H, d, J=7.8 Hz, H-1), 7.76 (2H, m, H-3, H-4), 7.74 (1H, dd, J=8.0 Hz, 1.0 Hz, H-7), 7.54 (1H, ddd, J=8.0 Hz, 7.3 Hz, 1.0 Hz, H-8), 7.44 (1H, m, H-2), 7.22 (1H, ddd, J=7.6 Hz, 7.3 Hz, 1.0 Hz, H-9), 4.37 (3H, s, N-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 156.18 (C-5a), 155.29 (C-6a), 137.02 (C-4a), 130.40 (C-3), 129.99 (C-1), 129.36 (C-8), 128.20 (C-10b), 128.13 (C-11), 123.94 (C-10a), 121.94 (C-2), 120.99 (C-10), 120.89 (C-11a), 119.92 (C-9), 117.69 (C-7), 114.16 (C-4), 33.10 (N-CH_3).

Compounds 3 and 4 were obtained by column chromatography on neutral Al_2O_3 of the total alkaloid fraction, prepared from the 80% ethanolic extract of dried root bark by a classical acid/base extraction procedure for alkaloids. Elution with $CHCl_3$/petroleum ether 2/1 yielded 3 as an amorphous orange powder. Subsequent elution with MeOH/$CHCl_3$ 1/9 resulted in the isolation of 4 as a green amorphous powder.

For compound 4, EIMS indicated a molecular weight of 462. However, the ^{13}C NMR spectrum only showed 8 aromatic CH groups, 7 quaternary carbons and a methyl group, while in the ^{1}H NMR spectrum, which was very similar to that of cryptolepine, the characteristic singlet due to H-11 was absent. This pointed towards a 11-substituted cryptolepine derivative. The high molecular weight (twice the molecular weight of cryptolepine minus 2H) led to the conclusion that 4 is the cryptolepine dimer 11-(cryptolepin-11-yl)-cryptolepine, or biscryptolepine, being a new alkaloid. Partial ^{1}H and ^{13}C NMR assignments were made by analogy with cryptolepine (1)[1], hydroxycryptolepine (2)[1] and cryptoquindoline (3) (see below).

Biscryptolepine (4, 11-(cryptolepin-11-yl)-cryptolepine, 9.3 mg), $C_{32}H_{22}N_4$; Rf = 0.42 (silica gel, $CHCl_3$/MeOH 7/3); EIMS (70 eV): M/z (relative abundance) 262 [M^+] (30), 448 (100), 434 (41), 433 (38), 231 (14), 216 (19), 104 (21); UV (λ_{max}, MeOH): 462, 378, 276, 225; UV (λ_{max}, 0.01 N KOH): 393, 375, 308, 282, 226; UV (λ_{max}, 0.01 N HCl): 462, 378, 275, 225; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.96 (2H, d, J=9.1 Hz, H-6 or H-4), 8.92 (2H, d, J=8.5 Hz, H-6 or H-4), 8.27 (2H, m, H-2*), 7.91 (2H, m, H-3*), 7.82 (4H, m, H-9, H-7*), 7.64 (2H, m, H-8*), 7.59 (2H, d, J=8.4 Hz, H-1), 5.38 (6H, s, N-CH_3) (* : assignments may be reversed); ^{13}C NMR (CD_3OD, 100 MHz): quaternary C: δ 148.51 (C-11), 141.34, 137.72 (C-4a, C-9a), 135.21, 127.15, 127.01 (C-5a, C-10a, C-11a), 115.91 (C-5b); aromatic CH: δ 136.27, 134.13, 129.75, 127.66, 127.41, 123.74, 119.54, 114.53 (C-1, C-2, C-3, C-4, C-6, C-7, C-8, C-9); δ 41.57 (N-CH_3).

For compound 3 EIMS indicated a molecular weight of 448, corresponding to a dimer of cryptolepine and quindoline. The ^{1}H and ^{13}C NMR spectra, being similar to those obtained for cryptolepine and quindoline, showed 17 aromatic CH groups, indicating a C-N linkage between the two units. A COSY experiment revealed four 4-proton spin systems. In a HSQC experiment the remaining singlet in the ^{1}H NMR spectrum (δ 7.59) was directly correlated to a ^{13}C NMR signal at δ 114.28, which is very similar to C-11 of quindoline (δ 115.22)[1]. This indicated that the quindoline moiety of the dimer was not substituted at C-11 or N-5, leading to the unequivocal conclusion that 3 was 11-(quindolin-10N-yl)-cryptolepine. The same compound was obtained previously from *C.sanguinolenta*, and named cryptoquindoline[4]. However, the NMR assignments were made by comparison with cryptolepine and quindoline only. We have reassigned some ^{1}H and ^{13}C NMR signals, based on COSY, HSQC and HMBC experiments.

Cryptoquindoline (3, 11-(quindolin-10N-yl)-cryptolepine, 14.2 mg), $C_{31}H_{20}N_4$; Rf = 0.03 (silica gel, $CHCl_3$/MeOH 7/3); EIMS (70 eV): M/z (relative abundance) 448 [M$^+$] (100), 434 (37), 241 (10), 224 (15), 218 (21); UV (λ_{max}, MeOH): 375, 338, 268, 228; UV (λ_{max}, 0.01 N KOH): 391, 341, 309, 269, 228; UV (λ_{max}, 0.01 N HCl): 365, 270, 223; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.70 (1H, d, J=7.6 Hz, H-6'), 8.33 - 8.43 (3H, m, H-4, H-6, H-4'), 7.88 (1H, m, H-3), 7.80 (1H, d, J=7.9 Hz, H-9), 7.72 (1H, d, J=8.5 Hz, H-1'), 7.64 - 7.68 (2H, m, H-1, H-3'), 7.59 (2H, H-8, H-11'), 7.40 - 7.49 (4H, m, H-2, H-2', H-7', H-8'), 7.17 (1H, m, H-7), 6.96 (1H, d, J=7.3 Hz, H-9'), 5.09 (3H, s, N-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 161.0 (C-9a), 146.89 (C-5'a)^a, 145.69 (C-9'a), 145.13 (C-4'a)^a, 142.55 (C-5a, C-10a (other signal not observed)), 134.87 (C-11), 133.57 (C-4a), 131.92 (C-8), 129.83 (C-10'a), 129.69 (C-8'), 129.42 (C-4'), 128.81 (C-3), 127.50 (C-1)^b, 127.11 (C-11'a), 126.74 (C-3')^b, 125.65 (C-1'), 125.24 (C-2)^c, 124.28 (C-2')^c, 123.72 (C-6), 123.20 (C-11a), 122.29 (C-6'), 121.72 (C-5'b), 121.36 (C-9), 121.36 (C-7'), 118.19 (C-7), 115.71 (C-4), 114.61 (C-5b), 114.28 (C-11'), 111.46 (C-9'), 38.17 (N-CH_3) (assignments with the same superscript may be reversed).

Acknowledgements

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