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ALKALOIDS OF *DYERA COSTULATA**

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Key Word Index—*Dyera costulata*; Apocynaceae; leaves; bisindole alkaloids.

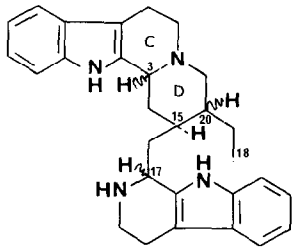
Abstract—Six bisindole alkaloids have been isolated from the leaves of *Dyera costulata* (Apocynaceae). One is the known ochrolifuanine A and the others are the novel ochrolifuanines E and F, and the 18-dehydroochrolifuanines A, E and F.

INTRODUCTION

Dyera costulata Hook F. [1] is an Apocynaceae from Malaysia. The genus contains two species which have never been investigated; their latex has been used in the rubber industry. *Dyera costulata* was later introduced in Zaire as an ornamental plant. The material used for the present study was collected in the Kinsantu Botanical Gardens (Zaire). It has been identified by H. Breyne; a herbarium specimen is deposited in the Brussels National Botanical Gardens under HB 3810.

RESULTS AND DISCUSSION

The usual extraction procedure [2] yielded 3.45 g crude alkaloidals per kg dried leaves. The mixture was separated by CC on alumina followed by prep. TLC. Six alkaloids were separated; they are, in order of increasing polarity: 18-dehydroochrolifuanine A(**2**) (4% of total), ochrolifuanine A(**1**) (9%), 18-dehydroochrolifuanine E(**4**) (3%), ochrolifuanine E(**3**) (5%), ochrolifuanine F(**5**) (1.6%) and 18-dehydroochrolifuanine F(**6**) (2.7%).



1 Ochrolifuanine A: 3α-H, 17β-H, 20β-H

2 18-Dehydroochrolifuanine A: Δ-18, 3α-H, 17β-H, 20β-H, C/D *trans*

3 Ochrolifuanine E: 3β-H

4 18-Dehydroochrolifuanine E: 3β-H, Δ-18

5 Ochrolifuanine F: 3β-H

6 18-Dehydroochrolifuanine F: 3β-H, Δ-18

Ochrolifuanine A(**1**) was identified by TLC (direct comparison with an authentic sample) and also by its optical rotation and CD data and UV, IR, ^{1}H NMR and mass spectra [3, 4]. The structure of the new alkaloid, **2** was established as 18-dehydroochrolifuanine A by spectral examination and chemical correlation. Its UV spectrum was typical of an indole. Its mass spectrum showed a molecular ion at *m/z* 436 ($C_{29}H_{32}N_4$) and fragments similar to those found in the mass spectrum of the ochrolifuanines [5]. The ^{1}H NMR spectrum of **2** displayed signals for a vinyl chain (three proton multiplet at δ 5.70–4.80) and three NH (singlets at δ 8.16, 7.96 and 1.90). The 3α-H-configuration and *trans* relationship between rings C and D are supported by the presence of Bohlmann bands in the IR spectrum and by a positive Cotton effect in the 280–300 nm region of the CD curve [6]. Finally, structure **2** was unambiguously proved by catalytical hydrogenation into a compound ($Y = 100\%$) in all respects identical with ochrolifuanine A(**1**).

Alkaloids **3** and **5**, the ochrolifuanines E and F, are isomers of ochrolifuanine A(**1**). All three alkaloids displayed similar mass spectra (M^+ 438, $C_{29}H_{34}N_4$) and very close UV, IR and ^{1}H NMR spectra. Similarly, alkaloids **4** and **6** are isomers of **2** (M^+ 436, $C_{29}H_{32}N_4$, vinyl chain on the ^{1}H NMR spectrum) and they were related to **3** and **5** by catalytical hydrogenation (**4** → **3**: 78%; **6** → 5: 88%). These alkaloids are thus 18-dehydroochrolifuanines E and F.

Differences between these alkaloids come from the configuration at C-3, C-17 and C-20, since C-15 is considered as a biogenetically invariant centre. The negative sign of the CD curve above 290 nm (Fig. 1) strongly favours a 3β-H configuration for alkaloids **3**–**6**. Whereas CD curves for **3** and **4** are superimposable, unexpected differences are found for **5** and **6** reflecting unexplained conformational differences between these two compounds. The available data do not allow the definitive assignment of the configuration at C-17 and C-20 for alkaloids **3**–**6**. Hemisyntheses are in progress to solve this problem.

If Pichon [1] wrote that there were intriguing similarities ('ressemblances troublantes") between *Dyera* and *Diplorrhynchus*, the chemical analysis described above is in favor of keeping these two genera separate.

*Part of the 'Thèse de Doctorat ès Sciences Pharmaceutiques', C. Mirand (1980) Reims No. 5. Plant samples were collected under the 'Etude Phytochimique de la Flore du Zaire' research project.

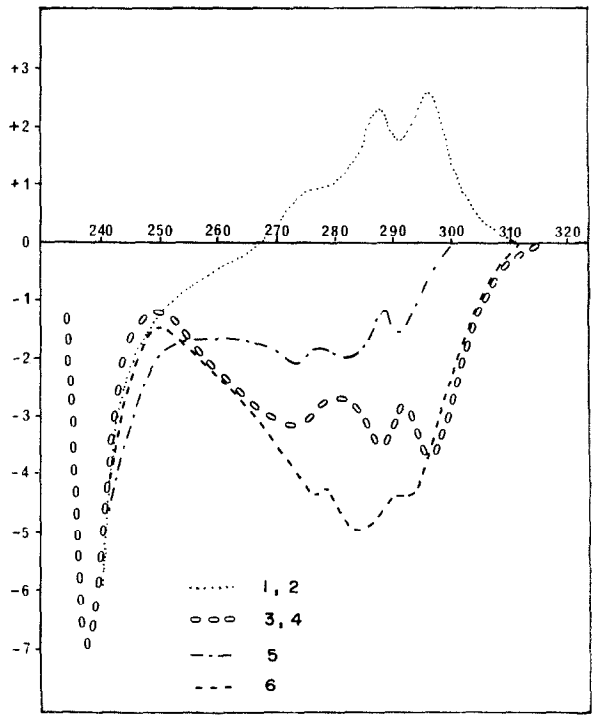


Fig. 1.

EXPERIMENTAL

General. NMR spectra were measured in $CDCl_3$ at 60 MHz on a WP60 Bruker spectrometer. Chemical shifts are given in δ-values with TMS as int. standard; coupling constants are given in Hz (*s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet; *m*, multiplet).

Extraction and isolation of alkaloids. The leaves (1.55 kg) were extracted in the usual fashion [2] to yield 5.35 g crude alkaloid mixture (3.45 g/kg). The mixture was placed on an alumina column (80 g) (Al_2O_3 standardized Merck, activity II–III) packed in C_6H_6 which was eluted in 64 ml fractions. Solvents were: C_6H_6 fr. 1–21; C_6H_6–Et_2O (99:1) fr. 22–28; (19:1) fr. 29–57; (9:1) fr. 58–73; (4:1) fr. 74–92; (1:1) fr. 93–108; Et_2O–MeOH (49:1) fr. 109–121; (9:1) fr. 122–130; (1:1) fr. 131–133. 18-Dehydroochrolifuanine A and ochrolifuanine A were in fr. 22–62; 18-dehydroochrolifuanine E and ochrolifuanine E were in fr. 71–95; ochrolifuanine F was in fr. 101–115; 18 dehydroochrolifuanine F was in fr. 111–119. The six alkaloids were then purified by prep. TLC.

Description of new alkaloids. 18-Dehydroochrolifuanine A(**2**): $C_{29}H_{32}N_4$ $[\alpha]_D + 4°$ (EtOH; *c* 0.7); UV λ_{max}^{EtOH} nm: 226, 283, 290; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 3420, 3260, 3060, 2840, 2800, 2750; MS *m/z* (rel. int.) 437, 436 $[M]^+$, 252, 251, 249, 185, 184, 171 (100), 170, 169, 156, 144, 143, 130; ^1H NMR: δ 8.16 (1H, *s*), 7.96 (1H, *s*), 7.60–6.90 (8H, *m*), 5.70–4.80 (3H, *m*), 4.20 (1H, *m*), 1.90 (1H, *s*). Ochrolifuanine E(**3**): $C_{29}H_{34}N_4$ $[\alpha]_D + 50°$ (EtOH; *c* 0.86); UV λ_{max}^{EtOH} nm: 227, 283, 290; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3470, 3410, 3260, 3060; MS *m/z* (rel. int.) 439, 438 $[M]^+$, 408, 265, 264, 252, 251 (100), 249, 223, 171, 156, 144, 143, 130; ^1H NMR: δ 9.00 (1H, *s*), 8.03 (1H, *s*), 7.70–6.90 (8H, *m*), 4.50–4.00 (2H, *m*), 2.25 (1H, *s*), 0.75 (3H, *t*, $J = 6$ Hz). Ochrolifuanine F(**5**): $C_{29}H_{34}N_4$ $[\alpha]_D$ $-37°$ (EtOH; *c* 0.92); UV λ_{max}^{EtOH} nm: 227, 283, 290; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 3440, 3300, 3070, 2810, 2760; MS *m/z* (rel. int.) 439, 438 $[M]^+$, 408, 265, 264, 252, 251 (100), 223, 171, 169, 156, 144, 143, 130; ^1H NMR: δ 7.85 (1H, *s*), 7.65 (1H, *s*), 7.70–6.90 (8H, *m*), 4.17 (1H, *m*), 1.83 (1H, *s*), 0.90 (3H, *t*, $J = 6$ Hz). 18-Dehydroochrolifuanine E(**4**): $C_{29}H_{32}N_4$ $[\alpha]_D + 80°$ (EtOH; c = 0.64); UV λ_{max}^{EtOH} nm: 228, 284, 291; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 3300, 3060; MS *m/z* (rel. int.) 437, 436 $[M]^+$, 406, 263, 250, 249 (100), 184, 171, 169, 156, 144, 143, 130; ^1H NMR: δ 9.10 (1H, *s*), 7.90 (1H, *s*), 7.70–6.80 (8H, *m*), 5.70–4.70 (3H, *m*), 4.50–4.00 (2H, *m*), 2.25 (1H, *s*). 18-Dehydroochrolifuanine F (**6**): $C_{29}H_{32}N_4$ $[\alpha]_D$ $-57°$ (EtOH; *c* 1.06); UV λ_{max}^{EtOH} nm 227, 283, 290; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 3430, 3280, 3060; MS *m/z* (rel. int.) 437, 436 $[M]^+$, 406, 263, 251, 250, 249 (100), 184, 171, 169, 156, 144, 143, 130; ^1H NMR: δ 8.03 (1H, *s*), 7.80 (1H, *s*), 7.70–6.90 (8H, *m*), 5.90–4.80 (3H, *m*), 4.50–4.00 (2H, *m*), 1.95 (1H, *s*).

Hydrogenation of 18-dehydroochrolifuanine A (**2** → **1**). 18-Dehydroochrolifuanine A(**2**) (14 mg) dissolved in EtOH (2 ml) was hydrogenated under normal pressure in the presence of 10% Pd–C (5 mg) for 5 hr. After filtration of the catalyst and evaporation of the solvent, the product obtained (14 mg) was identified as ochrolifuanine A(**1**) (TLC, $[\alpha]_D$, UV, IR, MS, ^1H NMR, CD).

Hydrogenation of 18-dehydroochrolifuanine E (**4** → **3**). In the same manner, **4** (13 mg) yielded 10 mg of a pure compound identical to **3** (TLC, $[\alpha]_D$, UV, IR, MS).

Hydrogenation of 18-dehydroochrolifuanine F (**6** → **5**). Compound **6** (25 mg) yielded 22 mg of a product identical to **5** (TLC, $[\alpha]_D$ UV, IR, MS, CD).

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REFERENCES

1. Pichon, M. (1948) *Mém. Mus. Nat. Hist. Nat. Nouv. Ser.* **27**, 190.

2. Petitfrère-Auvray, N., Verèauteren, J., Massiot, G., Lukacs, G., Sévenet, T. and Le Men-Olivier, L. (1981) *Phytochemistry* **20**, 1987.
3. Peube-Locou, N., Koch, M., Plat, M. and Potier, P. (1972) *Ann. Pharm. Fr.* **30**, 775.
4. Koch, M., Plat, M. and Préaux, N. (1973) *Bull. Soc. Chim. Fr.* 2868.
5. Préaux, N. (1976) Thèse de Doctorat, Paris VI.
6. Klyne, W., Swan, R. J., Dastoor, N. J., Gorman, A. A. and Schmid, H. (1967) *Helv. Chim. Acta* **50**, 115.