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AN ALKALOID FROM *CORYDALIS BUNGEANA*

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Key Word Index—*Corydalis bungeana*; Fumariaceae; alkaloid; (+)-13-epicorynoline; structural elucidation; X-ray diffraction.

Abstract—A new alkaloid, (+)-13-epicorynoline, was isolated from the whole plant of *Corydalis bungeana*. Its structure was established by ^{1}H NMR, mass spectroscopy, 2D-COSY-^{1}H NMR, UV, IR and the preparation of a derivative. The absolute configuration of the molecule was determined to be 11*R*,13*R*,14*S* by X-ray diffraction of its bromide salt.

INTRODUCTION

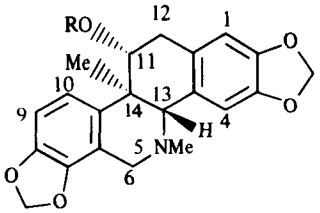
Corydalis bungeana Turcz. is used in north China as a febrifuge and detoxicant drug. Five alkaloids have already been obtained from this herb [1]. Further studies of the alkaloidal extracts were carried out in our laboratory. In this paper, the structure elucidation of a new minor alkaloid, 13-epicorynoline (**1**), is presented.

RESULTS AND DISCUSSION

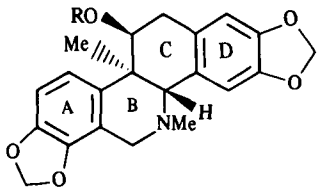
Alkaloid **1** showed a UV spectrum typical of corynolines (289 and 236 nm). The mass spectrum with a $[M]^+$ at *m/z* 367 (base peak) and fragments at *m/z* 349, 334, 318, 202, 190, 176 revealed a characteristic fragmentation pattern for the corynoline-group of alkaloids. The fragmentation pattern in the mass spectra of **1** and isocorynoline (**2**) were the similar [2], differing only in the abundances of some of the peaks. It was suspected, therefore, that **1** could be an isomer of isocorynoline (**2**).

Acetylation of **1** gave product **5**. The mass spectrum of **5** showed a $[M]^+$ at *m/z* 409 and fragments at *m/z* 394, 349 (base peak), 334, 321, 318, 202, 190, 176. The fragmentation pattern was the same as that of acetylisocorynoline (**6**) except for the different abundances of some of the peaks. This evidence gave further support to the suggestion of stereoisomerism.

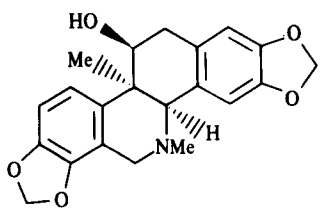
Studies of the ^{1}H NMR spectrum at 300 MHz ($CDCl_3$) coupled with a 2D-COSY ^{1}H NMR study (Fig. 1) of our new alkaloid allowed us to assign all the peaks in the spectrum as well as to derive structure **1** (Table 1). Two allylic type long-range couplings were observed between 1-H at δ6.63 and 12-H at δ2.82 and 3.10, 4-H at 7.12 and 14-H at δ3.97. One long-range coupling between 6-H at



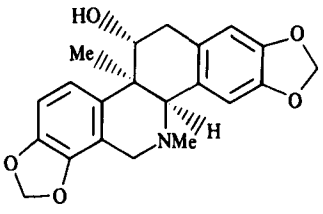
1 R = H
5 R = MeCO



2 Isocorynoline
R = H
6 Acetylisocorynoline
R = MeCO



3 Corynoline



4 11—Epicorynoline

Table 1. ^{1}H NMR spectral data of **1** and related compounds

C	1	2	3	4	5	6
HC_1	6.63 *s*	6.61 *s*	6.57 *s*	6.62 *s*	6.58 *s*	6.58 *s*
HC_4	7.12 *s*	7.17 *s*	6.65 *s*	6.63 *s*	7.26 *s*	7.24 *s*
	3.96 *d*	3.95 *d*	3.49 *d*	3.44 *d*	3.98 *d*	3.97 *d*
H_2C_6	(17)	(17)	(16)	(16)	(15)	(17)
	4.16 *d*	4.26 *d*	4.04 *d*	4.00 *d*	4.18 *d*	4.26 *d*
	(17)	(17)	(16)	(16)	(15)	(17)
HC_9	6.77 *d*	6.76 *d*	6.86 *d*	6.73 *d*	6.71 *d*	6.68 *d*
	(7)	(8)	(8)	(8)	(7)	(8)
HC_{10}	7.34 *d*	6.88 *d*	6.85 *d*	6.90 *d*	7.15 *d*	6.76 *d*
	(7)	(8)	(8)	(8)	(7)	(8)
HC_{11}	3.94 *dd*	4.32 *d*	3.99 *d*	4.53 *dd*	5.11 *dd*	5.49 *dd*
	(10, 7)	(5)	(3)	(10, 7)	(10, 7)	(5, 3)
	2.82 *dd*	2.82 *d*		2.60 *dd*	2.78 *dd*	2.81 *dd*
H_2C_{12}	(17, 10)	(18)	3.14 *d*	(18, 10)	(17, 10)	(18, 3)
	3.10 *dd*	3.21 *dd*	(3)	3.15 *dd*	3.23 *dd*	3.27 *dd*
	(17, 7)	(18, 5)		(18, 7)	(17, 7)	(18, 5)
MeC_{13}	1.19 *s*	1.10 *s*	1.13 *s*	1.10 *s*	1.29 *s*	1.18 *s*
HC_{14}	3.97 *s*	4.50 *s*	3.30 *s*	3.18 *s*	4.11 *d*	4.53 *s*
N-Me	2.43 *s*	2.48 *s*	2.18 *s*	2.16 *s*	2.46 *s*	2.51 *s*
MeCO–	—	—	—	—	2.16 *s*	1.76 *s*

Coupling constants (Hz) are in parentheses.

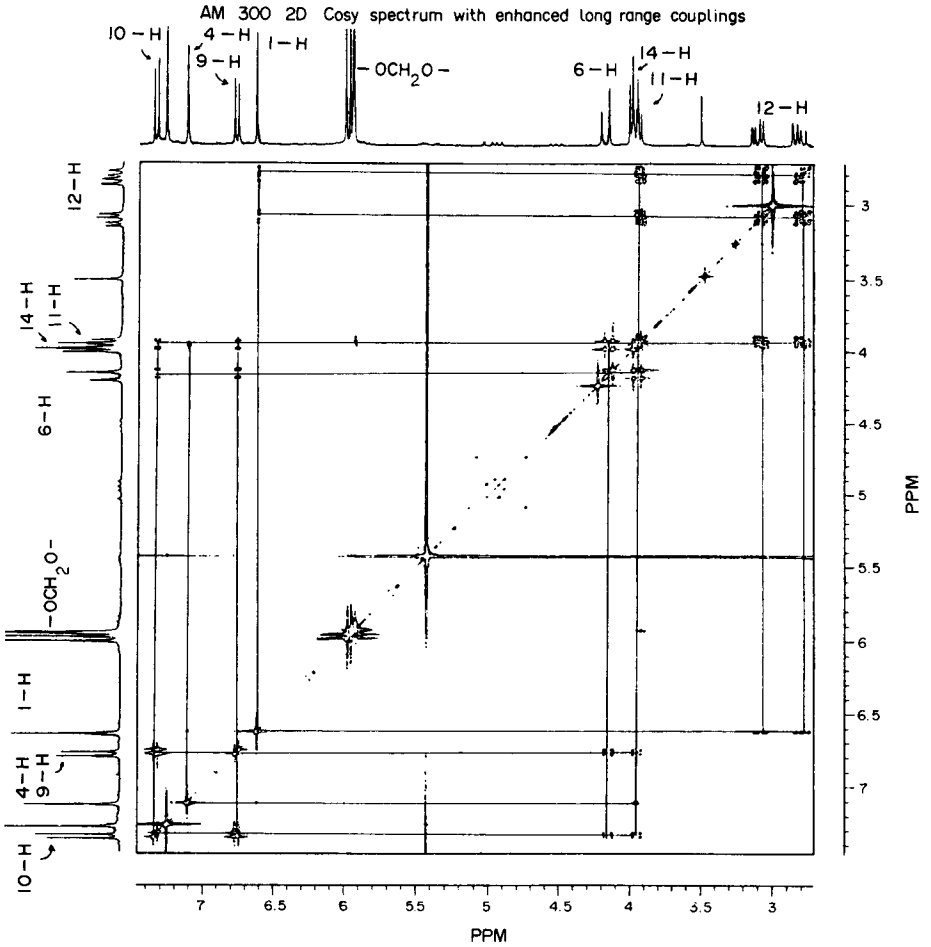


Fig. 1. 2D-COSY spectrum of **1**.

δ3.97 and 4.16 and 10-H at 7.34 was also observed. The long-range coupling between 6-H and 9-H at δ6.77 might be of an allylic type [3]. The coupling constants of all of these long-range couplings were too small to be detected even at 300 MHz. But they were evident in the 2D-COSY ^{1}H NMR study.

Comparative analyses of the ^{1}H NMR spectral data of **1** with those of three other known corynoline-group alkaloids namely isocorynoline (**2**), corynoline (**3**), and 11-epicorynoline (**4**) [2] allowed for the establishment of the stereostructure **1**. The signals of 4-H and N-Me at δ7.12 and 2.43 for alkaloid **1** and at δ7.26 and 2.46 for the acetylation product **5**, respectively, correlated well with isocorynoline (**2**) and acetylisocorynoline (**6**) with the *trans* junction of the B/C rings, but were different from those of corynoline (**3**) and 11-epicorynoline (**4**) which incorporate a *cis* junction of the B/C rings [4]. The chemical shift of the 9-H of alkaloid **1** was located at the same position as that of the other three known compounds, but the signal of 10-H appeared at δ7.34, further downfield than those for species **2**, **3** and **4**. This fact suggested that the configurations of 13-Me and 11-H of **1** were different. When the B/C rings are *trans* fused as indicated by the chemical shifts of 4-H and N-Me, there were only four possible configurations for 13-Me and 11-OH, i.e. $\alpha\alpha$, $\beta\beta$, $\alpha\beta$, $\beta\alpha$ with respect to 14β-H. As there already existed three known alkaloids with different configurations ($\alpha\beta$, $\beta\alpha$, $\beta\beta$ with respect to 14β-H for **2**, **3**, **4**). It was reasonable to deduce that 11-OH and 13-Me of alkaloid **1** had the $\alpha\alpha$ configurations.

An X-ray analysis of the bromide salt of **1** was carried out. The absolute configuration was determined by utilizing the anomalous scattering of bromine atoms. In the method the structure and its enantiomer were refined. The final X-ray model is shown in Fig. 2; the bond lengths are listed in Table 2. As can be seen, the B/C rings are *trans*-fused with half-chair/half-chair conformations and the dihedral angel between the A ring and B ring is *ca* 120°. The nitrogen atom and the neighbouring carbon atoms display a tetrahedral structure [112.3 (5), 107.1 (5), 112.1 (6)]. The absolute configuration of **1** was determined to be 11*R*,13*R*,14*S*.

A synthetic product named 13-epicorynoline had been reported [5]. However, the reported coupling constants between 11-H (δ4.25, *dd*, J = 8, 8 Hz) and 12-H (δ2.82, *dd*, J = 14.6, 9.8 Hz and δ2.89, *dd*, J = 15.2, 7.6 Hz) were irrelevant. Furthermore, the reported chemical shifts of

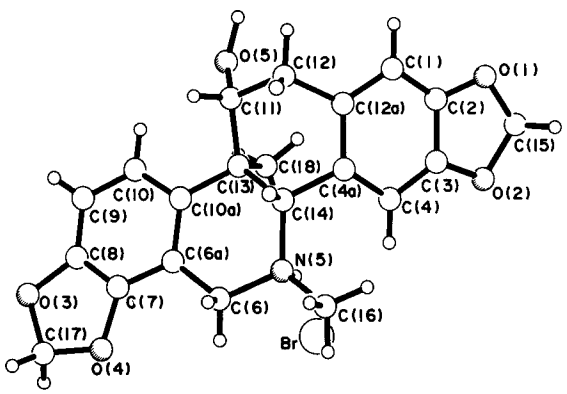


Fig. 2. View of the crystal structure of **1**.

Table 2. Bond lengths (A) of **1**

Atoms	Length	Atoms	Length
C1–C2	1.344 (7)	C7–04	1.383 (6)
C1–C12a	1.426 (6)	C8–C9	1.353 (8)
C2–C3	1.385 (8)	C8–03	1.403 (6)
C2–01	1.375 (6)	C9–C10	1.380 (7)
C3–C4	1.387 (6)	C10–C10a	1.408 (7)
C3–02	1.355 (6)	C10a–C13	1.504 (6)
C4–C4a	1.390 (6)	C11–C12	1.542 (7)
C4a–C12a	1.396 (7)	C11–C13	1.565 (7)
C4a–C14	1.499 (6)	C11–05	1.428 (7)
N5–C6	1.496 (5)	C12–C12a	1.481 (6)
N5–C14	1.497 (6)	C13–C14	1.565 (6)
N5–C16	1.515 (7)	C13–C18	1.539 (6)
C6–C6a	1.482 (7)	C15–01	1.408 (7)
C6a–C7	1.386 (6)	C15–02	1.432 (7)
C6a–C10a	1.405 (7)	C17–03	1.429 (9)
C7–C8	1.339 (8)	C17–04	1.396 (8)

N-Me (δ2.24) and 4-H (δ6.62) were located well out of the regions of δ2.40–2.52 and δ7.10–7.30, respectively, for those of corynolines possessing a *trans* junction of the B/C rings [4, 6].

The four compounds **1**–**4** have the same planar structure. There are three chiral carbons in the structure of each of them. Consequently eight stereostructures are possible for them; only four are diastereomers which would had different physical and chemical properties in achiral conditions. Three of the diastereomers isocorynoline **2**, corynoline **3**, 11-epicorynoline **4** have already been isolated before. The discovery of (+)-13-epicorynoline **1**, the only unknown diastereomer is a supplement to the chemistry of benzo(*c*)phenanthridine-type alkaloids.

EXPERIMENTAL

General. Mps are uncorr. UV spectra were measured in EtOH, IR spectra in KBr pellets. ^{1}H NMR were recorded at 300 and 400 MHz in $CDCl_3$ with TMS as int. ref. Low-pressure CC was carried out on silica gel (10–40 μ) and TLC on silica gel treated with NaOH (adsorbent: NaOH, 100:1).

Extraction. Plant material was collected from the eastern suburb of Beijing in 1985 and identified by Associate Profs Damu Yao and Dawen Zhao of our Institute. A voucher specimen has been deposited in the Department of Traditional Chinese Material Medica of our Institute.

Whole plant material (20 kg) of *C. bungeana* was refluxed twice in EtOH (200 l). Evapn of solvent under red. pres. left a thick residue. This residue was processed following usual acid-base work-up procedures and a non-phenolic base A (27 g) and a phenolic-base B (3.7 g) were obtained.

The non-phenolic base A (25 g) was sepd by low-pressure CC on 700 g silica gel (column size 5 × 100 cm). The column was gradiently eluted using cyclohexane with increasing amounts of EtOAc; three fractions were obtained. Fraction A_2 (cyclohexane–EtOAc, 3:2 to 2:3) was subjected to prep. TLC cyclohexane–Me_2CO, 4:1) to obtain alkaloid **1**.

Alkaloid **1** (+)-(13-epicorynoline). Colourless prisms, mp 231–232° ($CHCl_3$–MeOH). $[\alpha]_D^{25}$ = +136° ($CHCl_3$; *c* 0.26). UV λ_{max}^{EtOH} nm (log ε) 289 (4.06), 236 (4.10). ^{1}H NMR, ($CDCl_3$,

300 MHz), Table 1. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1610, 1490 (Ar). EIMS, 70 eV, *m/z* (rel. int.) 367 ($[M]^+$, 100), 349 (39), 334 (40), 318 (36), 202 (45), 190 (48), 176 (66). High resolution MS, *m/z*, 367.14139 ($C_{21}H_{21}NO_5$), 202.08689 ($C_{12}H_{12}NO_2$, M $-C_9H_9O_3$), 176.07200 ($C_{10}H_{10}NO_2$, $[M-C_{12}H_{12}O_3]^+$). Acetylation of **1** (Ac_2O–pyridine at 25° for 24 hr) gave the 11-acetate **5** (70% yield): $C_{23}H_{23}NO_6$. EIMS, 70 eV, *m/z* (rel. int.): 409 ($[M]^+$, 55), 394 (43), 349 (100), 334 (62), 321 (39), 318 (48), 202 (45), 190 (60), 176 (39). ^1H NMR, ($CDCl_3$, 400 MHz), Table 1. IR ν_{max}^{KBr} cm^{-1}: 1730, 1610, 1500, 1240, 1030.

Crystal data. The heavy atom derivative, bromo-alkaloid ($C_{21}H_{20}O_5$ NBr), was crystallized in the monoclinic space $P2_1$ with $a = 7.472(2)$, $b = 13.666(3)$, $c = 9.684(1)$ Å, $\beta = 104.10(1)°$, $Z = 2$. All unique diffraction maxima with $2\theta < 114°$ were collected on a R3m/E diffractometer using a graphite monochromater MoKα radiation (0.71069 Å) and variable speed 1°ω-scans. After correction for Lorentz Polarization and background effects, 1417 of 1419 unique reflections were judged and observed ($|Fo| > 3\sigma|Fo|$). The structure was solved using specific techniques for pseudosymmetry and the absolute configuration was determined utilizing the anomalous scattering of Br atoms. The final conventional crystallographic residual is 0.030 for the observed reflections. Full crystallographic data is available from the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Cambridge, UK.

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REFERENCES

1. Pang, H. and Fang, Q. (1981) *Yao Hsueh Teng Pao* **16**, 57.
2. Krane, B. D., Fagbul, M. O. and Shamma, M. (1984) *Lloydia* **47**, 1.
3. Jackman, L. M. and Sternhell (1969) *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* 2nd Edn. p. 313. Pergamon Press, New York.
4. Takao, N., Iwasa, K. and Kamigauchi, M. (1978) *Chem. Pharm. Bull.* **23**, 521.
5. Cushman, M., Abbaspour, A. and Gupta, Y. P. (1983) *J. Am. Chem. Soc.* **105**, 2873.
6. Hong, H. and Fang, Q. (1986) *Planta Med.* **3**, 193.