



# Coulteroberbinone, a quaternary isoquinoline alkaloid from *Romneya coulteri*

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Received 27 October 1998; received in revised form 27 October 1998; accepted 26 November 1998

## Abstract

Two quaternary isoquinoline alkaloids, benzyloquinolinium (+)-escholine and a new 13-oxo-tetrahydroprotoberberinium salt, were isolated from *Romneya coulteri* leaves. The trivial name (–)-coulteroberbinone was assigned to the new alkaloid. © 1999 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Romneya coulteri*; Papaveraceae; Coulteroberbinone; Isoquinoline alkaloids; Structure; Relative configuration

## 1. Introduction

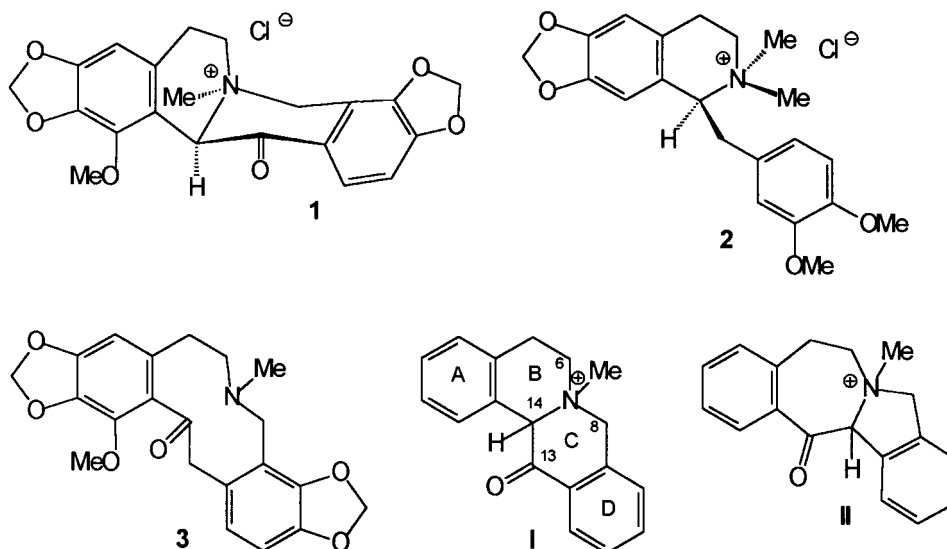
*Romneya coulteri* Harv. var. *trichocalyx* Jepson (Papaveraceae) roots contain protopine and coulteropine as main alkaloids (Stermitz, Chen, & White, 1966). The latter can be considered as the 1-methoxy-protopine; the additional oxygenated substituent is believed to be introduced at a later stage in the biosynthetic pathway (Battersby, Staunto, Wiltshire, Francis, & Southgate, 1975). In relation with this oxidation step, it was of interest to study the quaternary polar alkaloids in the plant. This paper reports on the isolation and structural elucidation of (–)-coulteroberbinone (**1**), the first *N*-methyl-13-oxo-tetrahydroprotoberberinium alkaloid found in nature. (+)-Escholine (**2**), the *N*-metho salt derived from (+)-romneine, was also characterized.

## 2. Results and discussion

(–)-Coulteroberbinone was obtained as an amorphous solid. Its FABHR mass spectrum gave the molecular formula  $C_{21}H_{20}NO_6^+$  and its EIMS revealed the

absence of a molecular ion, but fragments at  $m/z$  367  $[M-15]^+$  and  $m/z$  338  $[M-15-29]^+$  (base peak). The IR spectrum was consistent with the presence of a carbonyl group ( $1682\text{ cm}^{-1}$ ) and the UV spectrum with conjugation of this group with an aromatic ring (324 nm), as well as with a non-phenolic alkaloid. The  $^{13}\text{C}$  NMR spectrum of **1** was consistent with one carbonyl ( $\delta$  182.9), two methylenedioxy ( $\delta$  101.6 and 103.7) and one methoxy group ( $\delta$  59.6), in addition to three aromatic methines. In the aliphatic part of the spectrum, one methyl ( $\delta$  59.6), two methylene ( $\delta$  58.6 and 53.7) and one methine group ( $\delta$  70.0) were assigned to the four carbon atoms around the quaternary nitrogen. The  $^1\text{H}$  NMR spectrum revealed the presence of three aromatic protons, one as a singlet ( $\delta$  6.29) and the other two coupled in *ortho* ( $\delta$  7.60 and 6.86); the chemical shift of one of them suggested nearness to the carbonyl group. A low field aliphatic proton ( $\delta$  5.64), was easily exchanged with  $\text{D}_2\text{O}$  or  $\text{CD}_3\text{OD}$ , ( $[M]^+$  found: 383.13503;  $\text{C}_{21}\text{H}_{19}\text{DNO}_6^{\oplus}$  requires: 383.13533); it probably lies between the carbonyl group and the quaternized nitrogen. The aromatic substituents (one methoxy and two methylenedioxy groups) were tentatively arranged as in coulteropine (**3**), the main tertiary base reported for the plant (Stermitz et al., 1966). Hence, compound **1** was inferred to be a 1,2,3,9,10-substituted *N*-methyl tetra-

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hydroprotoberberinium (I) or an *N*-methyl isoindolo-benzazepinium salt (II) (Valencia et al., 1984). The observed three-bond correlation between the carbonyl carbon atom and the aromatic proton at position 12 in the HMBC spectrum is clearly consistent with the dibenzoquinolizidine skeleton of **1**. In addition, the two- and three-bond correlations found for the aromatic protons H-4 and H-11 confirms an aromatic substitution pattern identical with that in coulteropine (**3**). The HMBC spectrum was recorded in CDCl<sub>3</sub>–CD<sub>3</sub>OD and the rapid deuterium exchange of H-14 observed may account for its undetected correlation with the methoxy protons.

A *cis* B/C fused quinolizidinium salt was established as the relative stereochemistry of **1**. The assignment was derived from the low field *N*-methyl resonance in both <sup>1</sup>H and <sup>13</sup>C NMR spectra ( $\delta$  3.36 and 50.8 ppm, respectively) and the high field observed for C-6 ( $\delta$  53.7) (Iwasa, Sugiura, & Takao, 1982; Sariyar, Sary, Freyer, Guinaudeau, & Shamma, 1990). Further confirmation of the stereochemical assignment was obtained from the observed nOe between the *N*-methyl group and H-14 (Sariyar et al., 1990). This proton also exhibited nOe with one H-8, thus suggesting that both protons are pseudoaxial; consequently, the *N*-methyl group must be pseudoequatorial in the most stable conformation of **1**.

Thus, (–)-coulteroberbinone (**1**) is the first naturally occurring 13-oxo-tetrahydroprotoberberine *N*-metho salt with a *cis* B/C fused configuration that has been spectroscopically characterised to date.

The biosynthesis of 13-oxoprotopines has been shown to involve the sequence protoberberine → 13-hydroxyprotoberberine *N*-methosalt → 13-hydroxyprotopine → 13-oxoprotopine (Iwasa, Kamigauchi, Takao, & Cushman, 1993). The isolation of coulteroberbinone (**1**) suggests an alternative pathway in which full oxida-

tion at C-13 might occur before the oxidation at C-14 takes place, as suggested by Nalliah, Manske, and Rodrigo (1974) on the basis of the *in vitro* transformation of 13-oxoprotopines.

The second alkaloid isolated was characterised as a quaternary benzylisoquinoline. Its EIMS revealed the absence of molecular ion and the base peak at *m/z* 190, consistent with a methylenedioxy group on ring A. Two methoxy groups on the benzyl ring were inferred from the <sup>1</sup>H and <sup>13</sup>C NMR spectra. From this data, the alkaloid was identified as (+)-escholine (**2**) (Slavík, & Dolejs, 1973), the *N*-metho salt of the tertiary base (+)-romneine, also present in the plant rhizomes (Stermitz, & Teng, 1967). Full identification of **2** was accomplished by direct comparison with a synthetic sample.

### 3. Experimental

#### 3.1. General

M.p.'s: uncorr. EIMS: direct inlet, 70 eV. FABMS were obtained in the positive ion mode, using *m*-nitrobenzyl alcohol as matrix. <sup>1</sup>H and <sup>13</sup>C NMR signals were measured at 500 and 125 MHz, respectively. Proton chemical shifts are referred to the residual CHCl<sub>3</sub> ( $\delta$  7.24) signal and carbon chemical shifts to the solvent (<sup>13</sup>CDCl<sub>3</sub>  $\delta$  77). HMBC spectra were obtained by using standard programs. GF<sub>254</sub> silica gel was used for TLC.

#### 3.2. Plant material

*Romneya coulter* var. *trichocalyx* leaves were collected from La Cónsula Garden (Churriana, Málaga,

Table 1

<sup>1</sup>H and <sup>13</sup>C NMR assignments and three-bond HMBC correlations for **1**. Recorded in CDCl<sub>3</sub>–CD<sub>3</sub>OD; chemical shifts are given in ppm (δ)

H or C	<sup>1</sup> H (500 MHz)	<sup>13</sup> C (125 MHz)	HMBC ( <sup>3</sup> J) (carbon)
1	–	141.8s	
2	–	135.3s	
3	–	150.9s	
4	6.29s	102.1d	2, 5, 14a
4a	–	121.8s	
5	3.20m	23.5t	4, 14a
	2.84dd ( <i>J</i> =18.3, 6.3 Hz)		
6	3.8m, 3.5m	53.7t	4a, 14
8	5.26d ( <i>J</i> =15.7 Hz)	58.6t	6, 9, 12a, 14, NMe
	5.15d ( <i>J</i> =15.7 Hz)		
8a	–	111.7s	
9	–	144.9s	
10	–	154.3s	
11	6.86d ( <i>J</i> =8.3 Hz)	109.7d	9, 12a
12	7.60d ( <i>J</i> =8.3Hz)	124.9d	8a, 10, 13
12a	–	120.9s	
13	–	182.9s	
14	5.64s	70.0d	
14a	–	110.6s	
1-OMe	3.94s	59.6q	1
2,3-OCH <sub>2</sub> O	5.88d ( <i>J</i> =1.2 Hz)	101.5t2. 3	
	5.87d ( <i>J</i> =1.2 Hz)		
NMe	3.36s	50.8q	6, 8, 14
9,10-OCH <sub>2</sub> O	6.11d ( <i>J</i> =1.2 Hz)	103.7t9. 10	
	6.09d ( <i>J</i> =1.2 Hz)		

Spain). The plant was identified by Professor Baltasar Cabezudo and a voucher specimen is deposited in the herbarium of the Department of Plant Biology, Málaga (MGC 39485).

### 3.3. Extraction and isolation

Air-dried, powdered leaves (165 g) were exhaustively extracted with MeOH. The solvent was evaporated and the residue treated with 5% HCl and filtered. The filtrate was made alkaline with ammonium hydroxide (pH 11) and repeatedly extracted with CH<sub>2</sub>Cl<sub>2</sub>. The tertiary base-free aqueous solution was adjusted to pH 3–4 with conc. HCl and Mayer's reagent was added until precipitation ceased. The resulting precipitate was filtered, washed with H<sub>2</sub>O and suspended in MeOH. To this suspension, resin Amberlite® IRA-400 (Cl<sup>–</sup> form) was added until the precipitate was completely redissolved. The resin was filtered off and the clear solution concd to obtain 1.6 g of quaternary alkaloids as chlorides. Two major components were separated by prep. TLC (CHCl<sub>3</sub>–MeOH, 7:3): (–)-coultroberbinone (**1**, 576 mg) and (+)-escholinine (**2**, 464 mg).

### 3.4. Coultroberbinone (**1**)

White powder from MeOH–CHCl<sub>3</sub>–Et<sub>2</sub>O, m.p. 212–213°C. [ $\alpha$ ]<sub>D</sub> –53 (MeOH, 0.4). UV  $\lambda_{\max}$  nm (log  $\epsilon$ ) MeOH: 240 (4.19), 290 (3.95), 324 (3.75). IR  $\nu_{\max}$

cm<sup>–1</sup> (KBr): 1682 (C=O). <sup>1</sup>H and <sup>13</sup>C NMR (in CDCl<sub>3</sub>–CD<sub>3</sub>OD, see Table 1). EIMS *m/z* (rel int.): 367 [M]<sup>+</sup> of the demethylated base (37), 338 (100), 204 (14), 189 (22), 162 (21), 135 (35); FABHRMS: [M]<sup>+</sup> (found: 382.12914; C<sub>21</sub>H<sub>20</sub>NO<sub>6</sub><sup>+</sup> requires: 382.12906).

### 3.5. Escholinine (**2**)

[ $\alpha$ ]<sub>D</sub> +67 (MeOH, 0.22) [lit. [ $\alpha$ ]<sub>D</sub> +74 (MeOH, 0.31)] (Slavík, & Dolejš, 1973). IR, MS, <sup>1</sup>H and <sup>13</sup>C NMR compared positively with an authentic sample prepared by reaction of (+)-romneine with methyl iodide.

### Acknowledgements

The authors are greatly indebted to Professor Domingo Domínguez, of the University of Santiago de Compostela, for NMR measurements and helpful discussions. This work was financially supported by the Spanish DGICYT (PB94-1498).

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