Phytochemistry, 1972, Vol. 11, pp. 1467 to 1472. Pergamon Press. Printed in England.

STRUCTURES OF HOMOERYTHRINA ALKALOIDS FROM *CEPHALOTAXUS HARRINGTONIA**

R. G. POWELL

Northern Regional Research Laboratory†, Peoria, Ill. 61604, U.S.A.

(*Received* 26 *July* 1971)

Abstract—The structures of five minor alkaloids from *Cephalotaxus harringtonia* K. Koch var. *harringtonia* have been determined. These alkaloids are simple derivatives of schelhammericine (I). In alkaloid II, the methylenedioxy group of I is replaced by one hydroxyl group and one methoxyl group; alkaloid III is the analogous dimethoxy derivative. Alkaloids IV, V and VI are the corresponding C-3 epimers of alkaloids I, II and III. Though schelhammericine itself has not yet been observed in *Cephalotaxus*, alkaloids IV and V are identical in all respects with two alkaloids found in *Schelhammera pedunculata*. Alkaloids II, III and VI are previously unreported in the literature. The co-occurrence of these minor homoerythrina-type alkaloids (II–VI) with the major *Cephalotaxus* alkaloid cephalotaxine (VII) and its antileukemic esters (VIII–XI) is of interest biogenetically.

INTRODUCTION

RECENT investigations of *Cephalotaxus* extracts have revealed a number of new alkaloids[1,2] and several of these are of particular interest owing to their antitumor activity. A by-product of the isolation of quantities of these antileukemic alkaloids from *Cephalotaxus harringtonia* (Forbes) K. Koch var. *harringtonia* cv. Fastigiata[3] for further biological studies was the discovery of several minor alkaloids, the structures of which are reported in this paper. Particular emphasis is given to five homoerythrina alkaloids (II–VI). Although isolation of cephalotaxine (VII) and several related alkaloids (VIII–XII) is also described here, evidence for the total structures of VII–XI will be reported elsewhere.[4]

Alkaloids possessing the homoerythrina ring system are known in *Schelhammera pedunculata* F. Muell, in *Schelhammera multiflora* R. Br. [5–7] and in *Phelline comosa* Labill.[8] Cephalotaxine (VII), first described by Paudler *et al.*,[9] has been reported only in the genus *Cephalotaxus*.

* Presented at the 5th Great Lakes Regional Meeting of the American Chemical Society, Peoria, Ill., 10–11 June, 1971.

† A laboratory of the Northern Marketing and Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture.

[1] R. G. POWELL, D. WEISLEDER, C. R. SMITH, JR. and I. A. WOLFF, *Tetrahedron Letters* 4081 (1969).

[2] R. G. POWELL, D. WEISLEDER, C. R. SMITH, JR. and W. K. ROHWEDDER, *Tetrahedron Letters* 815 (1970).

[3] The entire trees were collected in Maryland during September 1968. The author acknowledges with thanks the receipt of the dried plant material from DR. ROBERT E. PERDUE, JR., U.S. Department of Agriculture, Beltsville, Md., in accordance with its program developed with the Cancer Chemotherapy National Service Center.

[4] References 1 and 2 give preliminary accounts of portions of this work.

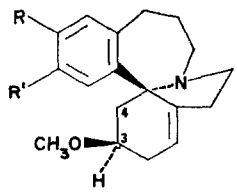
[5] J. S. FITZGERALD, S. R. JOHNS, J. A. LAMBERTON and A. A. SIOUMIS, *Austral. J. Chem.* **22**, 2187 (1969).

[6] S. R. JOHNS, J. A. LAMBERTON, A. A. SIOUMIS and H. SUARES, *Austral. J. Chem.* **22**, 2203 (1969).

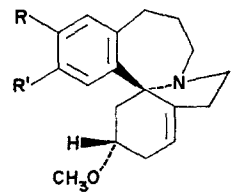
[7] S. R. JOHNS, J. A. LAMBERTON and A. A. SIOUMIS, *Austral. J. Chem.* **22**, 2219 (1969).

[8] N. LANGLOIS, B. C. DAS, P. POTIER and L. LACOMBE, *Bull. Soc. Chim. Fr.* 3536 (1970).

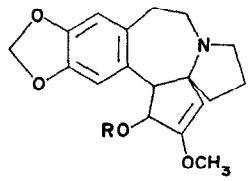
[9] W. W. PAUDLER, G. I. KERLEY and J. MCKAY, *J. Org. Chem.* **28**, 2194 (1963).



(I) R + R' = OCH_2O
(II) R + R' = OCH_3 + OH
(III) R = R' = OCH_3



(IV) R + R' = OCH_2O
(V) R = OCH_3; R' = OH
(VI) R = R' = OCH_3



(VII) R = H
(VIII) R = $C_{10}H_{17}O_5$
(IX) R = $C_{10}H_{17}O_5$
(X) R = $C_{11}H_{19}O_5$
(XI) R = $C_{10}H_{17}O_4$

RESULTS AND DISCUSSION

The NMR spectra of alkaloids I ($C_{19}H_{23}NO_3$), II ($C_{19}H_{25}NO_3$) and III ($C_{20}H_{27}NO_3$) are nearly identical except for the following features: I shows a two proton doublet at δ 5·84, assigned to a methylenedioxy group;[7] in the spectrum of alkaloid II this signal is absent and has been replaced by a three proton singlet at δ 3·75 assigned to an aryl methoxyl; although alkaloid III exhibits an additional methoxyl resonance at δ 3·82, its NMR spectrum is otherwise quite similar to the spectrum of II (Table 1). IR spectra of the three alkaloids demonstrate that only II contains a hydroxyl function.

TABLE 1. NMR DATA FOR SCHELHAMMERICINE (I) AND SOME RELATED *Cephalotaxus* ALKALOIDS*

Protons	Alkaloid					
	I†	II	III	IV	V	VI
H-1	5·52 m	5·53 m	5·54 m	5·51 m	5·52 m	5·55 m
H-4eq	—	—	—	2·77 q	2·74 q	2·78 q
H-4ax	1·77 q	1·78 q	1·80 q	1·57 t	1·60 t	1·58 t
H-15	6·83 s	6·84 s	6·89 s	6·73 s	6·77 s	6·74 s
H-18	6·54 s	6·60 s	6·56 s	6·61 s	6·63 s	6·65 s
OCH_2O	5·84 d	—	—	5·88 s	—	—
C3-OCH_3	2·73 s	2·66 s	2·68 s	3·22 s	3·22 s	3·19 s
Aryl-OCH_3	—	3·75 s	3·76 s	—	—	3·77 s
	—	—	3·82 s	—	3·86 s	3·83 s

* Measured in $CDCl_3$ with a Varian HA-100. Chemical shifts (δ) are expressed in parts per million from TMS.

† Schelhammericine (I) was received from Dr. J. A. Lamberton, CSIRO Chemical Research Laboratories, Melbourne, Australia, as a gift.

Alkaloids IV ($C_{19}H_{23}NO_3$), V ($C_{19}H_{25}NO_3$) and VI ($C_{20}H_{27}NO_3$) also give very similar NMR spectra. Alkaloid IV exhibits a signal at δ 5·88 due to a methylenedioxy group, alkaloid V has one aryl methoxyl (δ 3·86) and alkaloid VI contains two aryl methoxyl groups (δ 3·77 and 3·83). Of the three, only alkaloid V exhibits hydroxyl absorption in the IR.

Johns *et al.*[7] have shown that alkaloids of the schelhammericine series possess a methoxyl resonance in the NMR spectrum near δ 2·74. A quartet near δ 1·78 is attributed to the axial

proton at C-4. In the 3-*epi*-schelhammericine series, the methoxyl resonance appears near δ 3·17, and the H-4 axial signal appears as an apparent triplet near δ 1·52. Data in Table 1 indicate that alkaloids I–III belong to the schelhammericine series and that alkaloids IV–VI belong to the 3-*epi*-schelhammericine series.

Mass spectra of alkaloids I–VI further demonstrate their close structural relationship. Alkaloid IV exhibited all the major ions present in the published[7] mass spectrum of I with only minor differences in relative intensities. Similar observations were made when the mass spectra of either II and V or of III and VI were compared. Fragmentation patterns for all six alkaloids could be interpreted according to the scheme proposed by Johns *et al.*[7] Their scheme, based on the mass spectra of schelhammericine (I), 3-*epi*-schelhammericine (IV) and *Schelhammera* alkaloid B (V), was readily expanded to include *Cephalotaxus* alkaloids II, III and VI.

From physical measurements of alkaloids IV and V, including specific rotations, it is clear that these alkaloids from *Cephalotaxus* are identical in all respects to the ones isolated from *Schelhammera*. The NMR spectrum of IV and the published spectrum of *Schelhammera* alkaloid E[7] are indistinguishable. The absolute stereochemistry of IV and V is as shown since they have been related to schelhammerine;[5,7] the structure and absolute configuration of schelhammerine hydrobromide were determined by an X-ray crystal structure analysis.[10] *Cephalotaxus* alkaloids III and VI exhibit optical rotations of the same sign and magnitude as schelhammericine (I), $[\alpha]_D + 122°$ ($CHCl_3$), and alkaloids IV and V. Consequently, they may be considered to have the absolute configurations indicted. Similar reasoning applies for II, but uncertainty about magnitude of optical rotation, as well as the position of the aryl methoxyl in it, arises because the sample was small and not isolated in a high degree of purity. Apparently epimerization at C-3 has little effect on the optical rotation of these compounds.

The yields of all alkaloids isolated in this study from the combined stem and root materials are summarized in Table 2. Yields were comparable from the leaf alkaloids. The total homoerythrina alkaloids isolated from the stem and root sample amounted to less than 4% of the total alkaloid. Cephalotaxine was the most abundant alkaloid and was accompanied by significant amounts of several cephalotaxine esters (VIII–XI). Comparison

TABLE 2. YIELD OF VARIOUS ALKALOIDS FROM *Cephalotaxus harringtonia* STEMS AND ROOTS, 7·1 kg

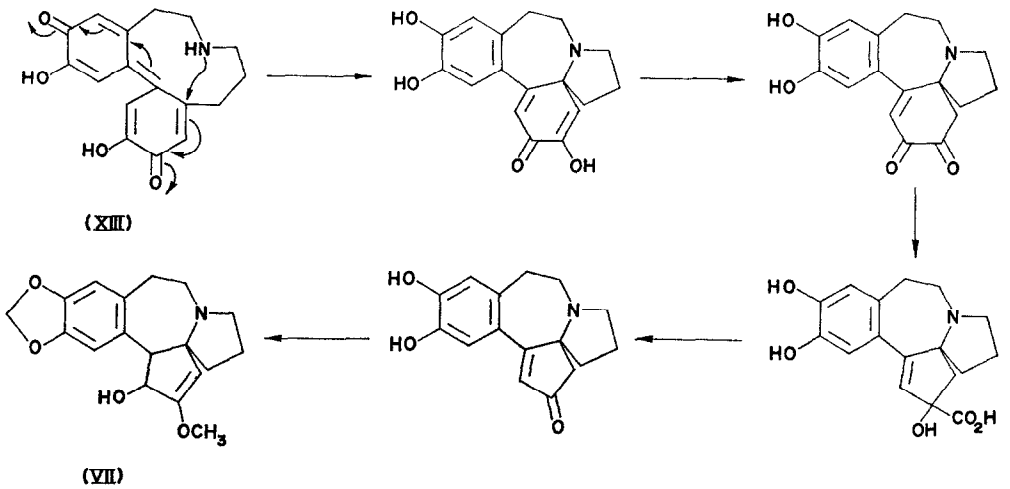
Alkaloid	Weight (g)	Total crude alkaloid (%)	Total plant material (%)
III	0·09	0·8	0·0013
IV*	0·13	1·1	0·0018
V	0·05	0·4	0·0006
VI	0·10	0·9	0·0014
Cephalotaxine (VII)	6·31	54·0	0·0860
Harringtonine (VIII)	0·47	4·0	0·0064
Isoharringtonine (IX)	0·77	6·1	0·0098
Homoharringtonine (X)	0·44	3·8	0·0061
Deoxyharringtonine (XI)	0·18	1·5	0·0024
Cephalotaxinone (XII)	0·02	0·2	0·0003
Others (including losses)	3·14	27·2	0·0439

* 3-*epi*-Schelhammericine.

[10] C. KOWALA and J. A. WUNDERLICH, *Z. Kristallogr.* **130**, 121 (1969).

of spectral properties of alkaloid XII with those reported for cephalotaxinone by McKay[11] indicates that the two are identical.

The observation that homoerythrina alkaloids occur in *Cephalotaxus*, along with the more predominant cephalotaxine and its derivatives, leads to some interesting biosynthetic speculations. Fitzgerald *et al.*[5] have proposed a pathway to the homoerythrina series



SCHEME 1. BIOSYNTHETIC ROUTE SUGGESTED FOR THE DERIVATION OF THE CEPHALOTAXINE SERIES OF ALKALOIDS.

utilizing derivatives of 1-phenylethyl-1,2,3,4-tetrahydroisoquinoline. An alternative ring closure of their proposed intermediate (XIII, Scheme I), followed by appropriate electron shifts, a benzilic acid-type rearrangement[12] and ultimate decarboxylation could then give rise to alkaloids of the cephalotaxine series. Such a sequence appears quite attractive as it allows both types of alkaloids to arise from the same precursors.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns block and are uncorrected. IR spectra were determined on a Perkin–Elmer Model 137 and UV spectra were recorded on a Beckman DK-2A spectrophotometer. Optical rotations were measured on a Cary Model 60 recording spectropolarimeter at 26° in 0·5-dm. cells. Mass spectral analyses were performed with a Nuclide 12-90G or with a CEC 21-492-1 spectrometer.

All compounds were analyzed by TLC on 0·25-mm Brinkman precoated Silica Gel F-254 plates (analytical) or 1-mm Silica Gel G plates (preparative) in the solvent system $CHCl_3$–MeOH (9:1), unless otherwise indicated. Analytical plates were visualized by exposure to iodine vapor and preparative plates were visualized by spraying with an alcoholic solution of bromothymol blue. Respective R_f values for the alkaloids in this solvent system are as follows: I, 0 66; II, 0·43; III, 0 59; IV, 0·77; V, 0·68; VI, 0·74; VII, 0 15; VIII, 0·30; IX, 0·50; X, 0·30; XI, 0·60; and XII, 0·84. When exposed to iodine, all spots initially gave a uniform brown color; upon standing in air for prolonged periods, the spots due to alkaloids I, II and III became greenish grey and the spots due to alkaloids IV, V and VI become grey-brown. Spots due to alkaloids VII–XI fade to a light yellow and eventually disappear. Because spots due to alkaloids VII–XI are elongated and are very sensitive to loading, R_f values for these compounds must be considered as approximate.

Extraction of the alkaloids. Dried roots of *C. harringtonia* (4 4 kg) were milled, placed in a percolator, along with 12 l. of 95% EtOH and allowed to stand overnight. The EtOH was then drained off and the plant material was extracted twice more with 8-l. portions of EtOH. Combined alcoholic extracts were evaporated under reduced pressure (below 40°) and the concentrate (200 ml) was diluted with 2·5 l. of 5% tartaric acid solution. The acidic solution was extracted repeatedly with $CHCl_3$ (discarding the $CHCl_3$

[11] J. B. MCKAY, Ph.D. Thesis, Ohio University, Athens, Ohio (1966).
[12] D. J. CRAM and G. S. HAMMOND, *Organic Chemistry*, 2nd edn., p. 499, McGraw-Hill, New York (1964).

extracts) and then made basic, to pH 9, by the addition of ammonia. Repeated extraction of the basic solution with $CHCl_3$ gave 9·5 g of crude alkaloids.

In a similar manner, 2·7 g of alkaloidal material was obtained from 3·4 kg of dried stems and, in a typical example, 3·2 g of alkaloid was isolated from 1·25 kg of leaf material. TLC of the stem and of the root alkaloids gave identical patterns; the leaf alkaloid showed some additional minor spots.

Separation of the alkaloids. The root and stem alkaloids were combined and 11·7 g of the mixture was separated into a series of fractions by countercurrent distribution in 10 tubes with 400 ml of each phase per tube. A $CHCl_3$ solution of the crude alkaloid mixture was placed in the first tube; $CHCl_3$ was used as the stationary phase and the mobile phase consisted of McIlvaine's buffer, pH 5. Alkaloids were recovered from the aqueous phase in each tube by adding ammonia to the buffer solution and extracting with $CHCl_3$. Approximate compositions of the fractions, as determined by TLC, were as follows: Tubes 1 and 2 (2·23 g) contained alkaloids III–VI, IX, XI and XII; tubes 3–7 (2·38 g) contained alkaloids VIII, IX and X; tube 8 (0·94 g) contained VII with some VIII and tubes 9 and 10 (5.77 g) contained relatively pure VII. Countercurrent fractionation of the leaf alkaloid (11·4 g) in an identical manner gave a similar series of components; however, some additional minor spots were evident in fractions 1–5.

The combined contents of tubes 1 and 2 (2·23 g) were chromatographed on 100 g of Brockman grade III neutral alumina. Collecting 20-ml fractions, the column was eluted successively with 200-ml portions of benzene, benzene–Et_2O (4:1), EtO_2, Et_2O–MeOH (20:1) and Et_2O–MeOH (4:1). Column fractions 5–10 were combined (169 mg) and subjected to preparative TLC on Silica Gel G plates that were developed with $CHCl_3$–MeOH (19:1). This procedure yielded two bands: The upper band, 92 mg, was a mixture of alkaloids IV and VI; the lower band yielded alkaloid III, 56 mg. In a similar manner, TLC of combined fractions 11–16 (371 mg) yielded cephalotaxinone (XII, upper band, 22 mg), 262 mg of a mixture of IV and VI and 109 mg of alkaloid III. Preparative TLC of column fractions 17–19 (92 mg) gave 51 mg of alkaloid V and preparative TLC of fractions 20–26 (254 mg) gave 180 mg of deoxyharringtonine (XI). Fractions 27–37 were combined to give 541 mg of isoharringtonine (IX) by preparative TLC.

Preparative TLC of countercurrent fractions 3–8 yielded 0·54 g of cephalotaxine (VII), 0·77 g of isoharringtonine (IX) and 1·16 g of an approximately 1:1 mixture of harringtonine (VIII) and homoharringtonine (X).[13]

Fractions containing alkaloids IV and VI were combined (354 mg) and chromatographed on 50 g of Brockmann grade III neutral alumina. The column was eluted with 600 ml of benzene followed by 600 ml of benzene–Et_2O (3:1); 20-ml fractions were collected. Column fractions 17–30 yielded 127 mg of alkaloid IV and fractions 37–40 contained 103 mg of alkaloid VI. The isolation of alkaloids III–XII is summarized in Table 2. Countercurrent distribution of the leaf alkaloids (11·4 g) in the manner described previously, followed by column chromatography of fraction 1 (2·43 g) and preparative TLC of combined column fractions 17–23 yielded 20 mg of alkaloid II. Only alkaloids V, VII and XII have been obtained crystalline. Because all alkaloids isolated except II gave single TLC spots and clean NMR spectra, no significant contaminants were present. The NMR data for alkaloids I–VI are summarized in Table 1.

Alkaloid II. Alkaloid II gave $[\alpha]_D$ + 76° (*c*, 0·40 in $CHCl_3$). Hydroxyl was evident, ν_{max} 3600 cm^{-1}, in $CHCl_3$. MS gave *m/e* 315 (M^+, 39%), 284 (27), 257 (base peak), 256 (33), 242 (19), 178 (77), 165 (23) and 146 (21).

Alkaloid III. Alkaloid III had $[\alpha]_D$ + 118 (*c*, 0·58 in $CHCl_3$); λ_{max} 281 (log ϵ 3·40), λ_{min} 259 nm (log ϵ 2·92) in EtOH; *m/e* 329 (M^+, 47%), 298 (36), 271 (base peak), 270 (46), 256 (20), 178 (75), 165 (31) and 146 (20). Found: M^+, *m/e* 329·2005; $C_{20}H_{27}NO_3$ requires 329·1990.

Alkaloid IV. Alkaloid IV had $[\alpha]_D$ + 123 (*c*, 0·51 in $CHCl_3$); λ_{max} 290 nm (log ϵ 3·64), λ_{min} 262 nm (log ϵ 2·92) in EtOH; *m/e* 313 (M^+, 43%), 282 (31), 255 (76), 254 (71), 178 (base peak), 165 (34) and 146 (23). Found: M^+, *m/e* 313·1676; $C_{19}H_{23}NO_3$ requires 313·1677.

Alkaloid V. Recrystallization from acetone gave alkaloid V as colorless needles, m.p. 150–152°, $[\alpha]_D$ + 115° (*c*, 0·36 in $CHCl_3$); λ_{max} 283 nm (log ϵ 3·51), λ_{min} 258 nm (log ϵ 2·78) in EtOH; ν_{max} 3600 cm^{-1} in $CHCl_3$; *m/e* 315 (M^+, 31%), 284 (28), 257 (74), 256 (70), 240 (16), 178 (base peak), 165 (40) and 146 (62). Found: M^+, *m/e* 315·1822; $C_{19}H_{25}NO_3$ requires 315·1834. Alkaloid V and *Schelhammera* alkaloid B[7] are identical in all respects.

Alkaloid VI. Alkaloid VI gave $[\alpha]_D$ + 122° (*c*, 0·50 in $CHCl_3$); λ_{max} 282 nm (log ϵ 3·48), λ_{min} 259 nm (log ϵ 3 02) in EtOH; *m/e* 329 (M^+, 38%), 298 (34), 271 (80), 270 (38), 256 (17), 178 (base peak), 165 (42) and 146 (26). Found: M^+, *m/e* 329·1992; $C_{20}H_{27}NO_3$ requires 329·1990.

Alkaloids VII–XII. Preliminary results[1,2] have been published concerning the structures of cephalotaxine (VII), $C_{18}H_{21}NO_4$; harringtonine (VIII), $C_{28}H_{37}NO_9$; isoharringtonine (IX), $C_{28}H_{37}NO_9$; and homoharringtonine (X), $C_{29}H_{39}NO_9$. Work on the cephalotaxine esters, including deoxyharringtonine (XI), $C_{28}H_{37}NO_8$, will be published elsewhere.[14]

[13] A mixture of VIII and X may be separated by a 200-tube countercurrent distribution using the system $CHCl_3$ McIlvaine's buffer, pH 5.

[14] K. L. MIKOLAJCZAK, R. G. POWELL and C. R. SMITH, JR., *Tetrahedron*, in press.

Alkaloid XII gave m.p. 170–178 (d); $[\alpha]_D$ −57° (*c*, 0·30 in $CHCl_3$); λ_{max} 290 nm (log ϵ 3·64), λ_{min} 270 nm (log ϵ 3·36), λ_{max} 243 nm (log ϵ 3·96) in EtOH; ν_{max} 1720 cm^{-1} in $CHCl_3$; *m/e* 313 (M^+, base peak), 298 (37%), 296 (26), 284 (17), 282 (16), 270 (21), 254 (30), 242 (16), 214 (18), 165 (81) and 137 (35). Found: M^+ *m/e* 313·133; $C_{18}H_{19}NO_4$ requires 313·131. The NMR spectrum shows one-proton singlets at δ 6·67, 6·60, 6·38 and 3·49; a two-proton singlet at δ 5·88 and a three-proton singlet at δ 3·78.

Acknowledgements—The author is indebted to Mrs. M. H. Rawls, Drs. D. Weisleder and W. K. Rohwedder, and Mr. R. Kleiman for technical assistance and spectral data; to Dr. R. B. Bates for discussions of the proposed biosynthetic pathway; and to Dr. C. R. Smith, Jr. for helpful suggestions.

Key Word Index—Cephalotaxus harringtonia; Cephalotaxaceae; homoerythrina; alkaloids; antileukemic agents.