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DESMETHYLCEPHALOTAXINONE AND ITS CORRELATION WITH CEPHALOTAXINE

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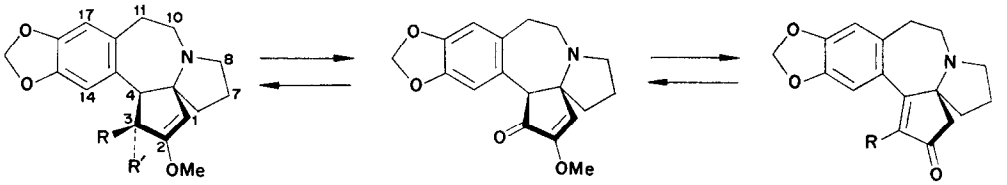
Key Word Index—*Cephalotaxus harringtonia*; Cephalotaxaceae; alkaloids; cephalotaxine; cephalotaxinone; desmethylcephalotaxinone.

Abstract—A new alkaloid, desmethylcephalotaxinone (IIIa) has been isolated from *Cephalotaxus harringtonia* (Forbes) K. Koch var. *harringtonia* cv. Fastigiata. Its structure has been established by spectral methods and by a partial synthesis from cephalotaxine (Ia) via cephalotaxinone (II). Methylation of IIIa with dimethoxypropane yields II along with some isocephalotaxinone (IIIb). Compound II was confirmed as a naturally occurring minor *Cephalotaxus* constituent. Reduction of II with sodium borohydride proceeds stereospecifically to give Ia.

INTRODUCTION

THE FIRST definitive work on alkaloids of the genus *Cephalotaxus* was carried out by Paudler *et al.*[1] and by McKay.[2] Investigations by other workers since have resulted in assignment of structure Ia to cephalotaxine;[3,4] cephalotaxine usually accounts for more than 50% of the total alkaloid present in *Cephalotaxus* extracts. Four lesser *Cephalotaxus* alkaloids, all closely related esters of cephalotaxine, possess marked activity against



(Ia) R = OH; R′ = H
(Ib) R = H; R′ = OH

(II)

(IIIa) R = OH
(IIIb) R = OMe
(IIIc) R = OAc

experimental leukemia in mice.[5,6] This activity prompted detailed investigations of alkaloids present in *C. harringtonia*[7] and *C. wilsoniana*.[8] It is now clear that homoerythrina alkaloids are also present in *Cephalotaxus* extracts and that cephalotaxinone usually occurs as a

* Agricultural Research Service, U.S. Department of Agriculture.

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[8] POWELL, R. G., MIKOLAJCZAK, K. L., WEISLEDER, D. and SMITH, JR., C. R. (1972) *Phytochemistry* **11,** 3317.

minor constituent of *C. drupacea*[2] and *C. harringtonia*.[7] We now wish to report the isolation of desmethylcephalotaxinone (IIIa) and to describe some of the chemistry of IIIa and several related *Cephalotaxus* alkaloids.

RESULTS AND DISCUSSION

It was recognized earlier[7] that considerable losses occurred when certain *Cephalotaxus* alkaloid fractions were chromatographed on neutral alumina. Further elution of the columns with dilute aqueous acetic acid has revealed a new alkaloid, desmethylcephalotaxinone (IIIa). Alkaloid IIIa melted at 102–107° and gave a plain positive ORD curve in the range 600–450 nm, $[\alpha]_D$ +2·3°. The IR spectrum of IIIa in $CHCl_3$ showed vinylic hydroxyl (3520 cm^{-1}) and carbonyl (1690 cm^{-1}) peaks. NMR of IIIa (Table 1) revealed the presence of a methylenedioxy group (2-proton *s*, δ 5·91), two aromatic protons (*s* δ 6·90, δ 6·63) and two protons attributed to an isolated methylene group (*s* δ 2·54, $CDCl_3$). However, the two isolated methylene protons appear as an *AB* quartet centered at δ 2·38 in DMSO-d_6 solution. A MS of IIIa revealed a strong molecular ion (base peak) at *m/e* 299. Assuming that IIIa had an empirical formula of $C_{17}H_{17}NO_4$ (MW 299) and that it possessed the same ring system as cephalotaxine, we concluded that IIIa has the structure shown.

TABLE 1. NMR DATA FOR CEPHALOTAXINE (Ia), EPICEPHALOTAXINE (Ib), CEPHALOTAXINONE (II), DESMETHYLCEPHALOTAXINONE (IIIa), ISOCEPHALOTAXINONE (IIIb) AND ACETYLDESMETHYLCEPHALOTAXINONE (IIIc)*

Protons	Alkaloid Ia	Ib†	II	IIIa	IIIb	IIIc
H-1	4·89s	4·79s	6·38s	2·54s‡	2·49s	2·59s
H-3	4·70d	4·56d	—	—	—	—
H-4	3·63d	3·06d	3·49s	—	—	—
$J_{3,4}$	9·5Hz	5·0Hz	—	—	—	—
H-14	6·65s	6·64s	6·67s	6·90s	6·72s	6·68s
H-17	6·61s	6·59s	6·60s	6·63s	6·65s	6·59s
Aryl–OCH_2O–	5·86s	5·82s	5·88s	5·91s	5·94s	5·93s
Vinyl–OMe	3·70s	3·64s	3·78s	—	3·87s	—
O ‖ –OCMe	—	—	—	—	—	2·15s

* Measured in $CDCl_3$ with a Varian HA-100. Chemical shifts (δ) are expressed in ppm from tetramethylsilane.

† K. L. Mikolajczak, in preparation.

‡ In DMSO-d_6, the two H-1 protons of alkaloid IIIa exhibit slightly different chemical shifts; in this solvent they appear as an *AB* quartet centered at δ 2·38 (rather than the 2-proton *s* observed at δ 2·54 in $CDCl_3$).

Upon acetylation, IIIa gave the corresponding vinylic acetate (IIIc). The NMR spectrum of IIIc contained peaks at δ 6·68 and 6·59 (2 aromatic protons), δ 5·93 (2-proton *s*, methylenedioxy), δ 2·59 (2-proton *s*, isolated methylene) and δ 2·15 (3-proton *s*, vinylic acetate moiety). A MS of IIIc gave a strong molecular ion (98%) at *m/e* 341 and a base peak at *m/e* 299. In the *m/e* 30–300 region the MS of IIIc was quite similar to the MS of IIIa. Thus

the major fragmentation appears to be loss of ketene (C_2H_2O); loss of ketene may be expected when vinylic and phenolic acetates decompose.[9,10]

A partial synthesis of IIIa was realized, starting from cephalotaxine (Ia), and this transformation served to verify the structure of IIIa. Oxidation of Ia, using the modified Oppenauer oxidation as described by McKay,[2] gave II in good yield. Alkaloid II prepared in this manner was identical to natural cephalotaxinone in all respects. Cleavage of the enol ether linkage of II was achieved by vigorous hydrolysis with aq. HCl. Less than 30% hydrolysis was observed after 3 hr at 80°. The possibility that IIIa was an artifact derived from cephalotaxinone is remote as the isolation of natural IIIa was done using tartaric acid (pH 2 or above) at temperatures less than 40°. Except for optical rotation, the hydrolysis product was identical in all respects to natural IIIa, and therefore, the structure of IIIa, as indicated, was confirmed. Alkaloid IIIa obtained by hydrolysis of natural II gave a markedly higher optical rotation than natural IIIa ($[\alpha]_D$ +40·0° as compared with +2·3°). Evidently, natural IIIa was isolated as a nearly complete racemate. Alkaloid IIIa is apparently the most stable of the three possible tautomeric forms that might be expected from acid hydrolysis of II; presumably this stability is due to extended conjugation with the aromatic ring.

Further support for the structure assigned to IIIa was obtained by converting this compound to Ia. Compound IIIa was first converted to II by using 2,2-dimethoxypropane in acidic solution. Alkaloid II, which had been prepared by methylation of natural IIIa, was optically inactive; lack of optical activity for II from this reaction may be explained by the earlier observation that the starting material, natural IIIa, was nearly racemic. The reaction appears to give only the natural epimer at C-4. A minor methylation product was identified as the isomeric ether IIIb. The NMR spectrum of IIIb was distinguished by a 2-proton singlet at δ 2·49 (isolated methylene group), and by a vinyl methoxyl signal at δ 3·87. To complete the interconversion of Ia and IIIa, a sample of natural II was reduced with sodium borohydride to give Ia in nearly quantitative yield. The NMR spectrum of the crude reduction product gave no evidence that any of the alternative reduction product, epicephalotaxine (Ib), was formed. The NMR spectrum of alkaloid Ib differs markedly from that of Ia in the chemical shifts and coupling constants of the H-3 and H-4 proton signals and also in the position of the H-1 and vinyl methoxyl signals (Table 1). McKay[2] isolated Ia as the major product and Ib as a minor product when he reduced II with lithium aluminum hydride. These reagents give preferential hydride attack from the least hindered side of the carbonyl, and this attack leaves the resulting hydroxyl in the more hindered position.

Total synthesis of racemic alkaloids Ia, II and IIIa was recently achieved by Auerbach and Weinreb.[11] Comparison of their synthetic alkaloids with our corresponding natural materials showed them to be identical in all respects, except for optical activity and minor differences in melting points. Another synthesis of Ia and II has also been reported by Semmelhack *et al.*[12]

A plausible explanation for the presence of cephalotaxinone and desmethylcephalotaxinone in *Cephalotaxus* extracts is to assume that they are intermediates in the bio-

[9] Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1964) *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. II, pp. 206–208, Holden-Day, San Francisco.
[10] Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1967) *Mass Spectrometry of Organic Compounds* p. 471, Holden-Day, San Francisco.
[11] Auerbach, J. and Weinreb, S. M. (1972) *J. Am. Chem. Soc.* **94,** 7172.
[12] Semmelhack, M. F., Chong, B. P. and Jones, L. D. (1972) *J. Am. Chem. Soc.* **94,** 8629.

synthesis of cephalotaxine. If the actual biosynthesis of Ia proceeds via methylation of IIIa followed by reduction of II, this pathway would be consistent with the general biosynthetic route suggested earlier.[7]

EXPERIMENTAL

General. M.ps were determined on a Fisher-Johns block and are uncorrected. IR analyses were done on 1% solutions in $CHCl_3$. Optical rotations were determined on a Cary Model 60 recording spectropolarimeter in 0·5-dm cells. Mass spectral analyses were performed with a Du Pont (CEC) 21-492-1 spectrometer. NMR spectra were measured with a Varian HA-100 in $CDCl_3$ solution, unless otherwise specified. All compounds were analyzed by TLC with appropriate solvent systems on Brinkman precoated 0·25-mm Silica Gel F-254 plates. Spots were visualized either by staining the plates with I_2 vapor or by spraying them with an ethanolic solution of bromothymol blue. All preparative separations were made on 1-mm Silica Gel G layers with bromothymol blue visualization. $CHCl_3$ extracts were routinely dried over Na_2SO_4 or $MgSO_4$.

Separation of the alkaloids. Typical isolation procedures for a number of *Cephalotaxus harringtonia* alkaloids have been described elsewhere.[7] Five *Cephalotaxus* trees[13] (76 kg) yielded 35 g of crude alkaloid (0·05%). A 34-g portion of the crude alkaloid mixture was separated into a series of fractions by countercurrent distribution in 10 tubes with 1·6 l. of each phase per tube. A $CHCl_3$ solution of the alkaloid mixture was placed in the first tube; $CHCl_3$ served 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 (3·7 g) contained a mixture of homoerythrina alkaloids, deoxyharringtonine, isoharringtonine, harringtonine, homoharringtonine and cephalotaxinone (II). Tubes 3–8 (5·1 g) contained cephalotaxine, harringtonine, homoharringtonine and isoharringtonine; tubes 9 and 10 (21·2 g), relatively pure cephalotaxine (Ia). The various alkaloids were separated by a combination of column chromatography, on Brockmann grade-III neutral alumina and TLC. This procedure gave the following yields of alkaloids isolated from countercurrent distribution tubes 1–4: Cephalotaxinone (II), 0·4 g; deoxyharringtonine, 0·3 g; a mixture of harringtonine and homoharringtonine, 0·2 g; isoharringtonine, 1·5 g; and a total of 2·2 g of several homoerythrina alkaloids. Tubes 5–7 yielded 0·2 g isoharringtonine, 1·4 g of a mixture of harringtonine and homoharringtonine and 0·2 g of unidentified materials. Tube 8 gave 0·4 of a mixture of harringtonine and homoharringtonine along with 0·9 g cephalotaxine (Ia). Tubes 9 and 10 (21·2 g) showed only one spot when examined by TLC and, after column chromatography and recrystallization from Et_2O, gave 16·2 g of pure crystalline cephalotaxine (Ia). Only 1·8 g of alkaloid was recovered from 2·9 g of alkaloid chromatographed (CCD tubes 5–7) on an alumina column even after prolonged elution with MeOH–$CHCl_3$ (1:3). Consequently, the column was further eluted with 5% aq. HOAc (500 ml). When the aq. eluate was made basic with NH_4OH and repeatedly extracted with $CHCl_3$, an additional 0·2 g of alkaloidal material was obtained. TLC of this material showed a single spot, and NMR demonstrated that it was a previously unrecognized alkaloid, desmethylcephalotaxinone (IIIa).

Cephalotaxine (Ia). Recrystallization from Et_2O gave cephalotaxine, m.p. 135–136° $[\alpha]_D^{26°}$ −188° (*c* 0·50, $CHCl_3$) which was identical in all respects (IR, UV, NMR and MS) with authentic samples of cephalotaxine reported previously.[5] The NMR spectrum of Ia is recorded in Table 1.

Cephalotaxinone (II). Cephalotaxinone, after several recrystallizations from Et_2O, gave m.p. 195–200° (d), $[\alpha]_D^{26°}$ − 139° (*c* 0·54, $CHCl_3$); λ_{max} 290 nm (log ϵ 3·69), λ_{min} 270 nm (log ϵ 3·46), λ_{max} 242 nm (log ϵ 4·00), λ_{min} 222 nm (log ϵ 3·78) in EtOH; ν_{max} 1720 cm^{-1} in $CHCl_3$. The mass and NMR spectra of II were indistinguishable from the respective spectra of a sample of II reported earlier.[7] The higher m.p. and specific rotation for II, we observed in our present study, are evidence of a higher state of purity.

Natural desmethylcephalotaxinone (IIIa). Recrystallization from MeOH gave IIIa as a pale yellow solid, m.p. 102–107° $[\alpha]_D^{26°}$ + 2·3° (*c* 0·52, MeOH), λ_{max} 317 nm (log ϵ 4·01), λ_{min} 270 nm (log ϵ 3·71) in EtOH; ν_{max} 3520 cm^{-1} (hydroxyl) and 1690 cm^{-1} (carbonyl) in $CHCl_3$; *m/e* 299 (M^+, 100%), 282(16), 270(10), 257(16), 256(59), 242(11) and 288(27). The NMR spectrum of IIIa is given in Table 1.

Acetyldesmethylcephalotaxinone (IIIc). A 102-mg portion of IIIa was dissolved in 6 ml Ac_2O–pyridine (1:1) and allowed to stand overnight. After excess solvent was evaporated under reduced pressure, the oil remaining was taken up in a mixture of $CHCl_3$ (5 ml) and H_2O (20 ml). The mixture was basified with NH_4OH and then extracted 4 × with 5-ml portions of $CHCl_3$. The combined chloroform extracts yielded 103 mg of crude product. The product was separated by preparative TLC (one plate, developed with 5% MeOH in $CHCl_3$) yielding 54 mg of IIIc. Alkaloid IIIc gave strong bands at ν_{max} 1765, 1705, 1475, 1370, 1315, 1085 and 1040. The MS of IIIc gave prominent ions at *m/e* 341 (M^+, 98%), 300(20), 299(100), 298 (21),

[13] Five entire trees, *Cephalotaxus harringtonia* (Forbes) K. Koch var. *harringtonia* cv. Fastigiata, were collected in Oregon during August 1969.

282(16), 271(21), 270(16), 257(18), 256(47), 242(19) and 228(30). The NMR spectrum of IIIc is summarized in Table 1.

Oxidation of cephalotaxine (Ia). A solution of 0·5 g Ia, 0·8 g benzophenone, 0·1 g potassium *t*-butoxide and 50 ml *t*-butyl alcohol was refluxed for 7 hr. Excess solvent was removed under N_2 on a steam bath. The residue was dissolved in 5% aq. HOAc and the solution extracted with $CHCl_3$ to remove acidic and neutral materials. The remaining aqueous solution was made basic with NH_4OH and again extracted with $CHCl_3$. The latter $CHCl_3$ extracts gave 297 mg of material which, after purification by preparative TLC, yielded 232 mg of II. Alkaloid II prepared in this manner gave m.p. 172–195° (d), $[\alpha]_D^{26°}$ −146° (*c* 0·59, $CHCl_3$); λ_{max} 290 nm (log ϵ 3·64), λ_{min} 270 nm (log ϵ 3·42), λ_{max} 242 nm (log ϵ 3·96), λ_{min} 222 nm (log ϵ 3·75). The sample was otherwise identical in all respects (IR, NMR and MS) with II of natural occurrence.

Hydrolysis of cephalotaxinone (II). Preliminary experiments demonstrated that less than 30% hydrolysis occurred when II was allowed to stand in a 1·0 N HCl solution at 80° for 3 hr. A 151-mg sample of II, in 30 ml of 1·0 N HCl, was refluxed for 21 hr. The solution was then made basic with NH_4OH and extracted 5× with 15-ml portions of $CHCl_3$. The combined $CHCl_3$ extracts yielded 135 mg of hydrolysis product, which gave a single TLC spot, m.p. 104–110°, $[\alpha]_D^{26°}$ +40·0° (*c* 0·43, MeOH). IR, NMR and MS of the product were indistinguishable from the corresponding spectra of natural IIIa. A portion of IIIa, obtained by hydrolysis of II, was acetylated and the acetylation product was identical in all respects (IR, NMR and MS) with IIIc prepared from naturally occurring IIIa.

Methylation of desmethylcephalotaxinone (IIIa). A 180-mg sample of desmethylcephalotaxinone was dissolved in 10 ml anhyd. C_6H_6; 4 ml 2,2-dimethoxypropane and 3 ml 1 N H_2SO_4 in MeOH were added; and then the solution was refluxed under N_2 for 8 hr. After being left to stand at room temp. overnight, the solution was basified with Na_2CO_3 solution and extracted with $CHCl_3$; yield, 450 mg. TLC on silica with MeOH–$CHCl_3$ (15:85) revealed a number of spots, but the major one, R_f 0·86, was not basic to bromothymol blue. This crude product was dissolved in $CHCl_3$ and washed with aqueous tartaric acid solution. Basification of the acid solution followed by extraction with $CHCl_3$ yielded 90 mg of crude product that showed three major spots, R_f 0·40 (starting material), 0·69 and 0·77. Preparative TLC on silica with MeOH–$CHCl_3$ (1:19) yielded 13 mg of the R_f 0·77 spot, which was identical by IR, NMR and MS to natural cephalotaxinone. It also gave m.p. 177–184°, $[\alpha]_D^{26°}$ 0° (*c* 0·13, $CHCl_3$), and UV λ_{max} 290 nm (log ϵ 3·52), λ_{min} 270 nm (log ϵ 3·30), λ_{max} 242 nm (log ϵ 3·85) and λ_{min} 222 nm (log ϵ 3·71). The R_f 0·69 spot (IIIb, 9 mg) had IR 1685 (–C=O), 1590, 1615, 1105, 1035 and 930 cm^{-1} (methylenedioxy); NMR shown in Table 1; MS *m/e* 313(M^+, 100), 298(38), 285(18), 282(10), 271(36), 257(30), 242(20) and 228(12).

Reduction of cephalotaxinone (II). A 41-mg sample of cephalotaxinone was dissolved in 8 ml 95% EtOH and cooled in an ice bath. Na BH_4 (38 mg) dissolved in 4 ml EtOH was added to the cold alkaloid soln. dropwise over 10 min, the ice bath was removed, and the soln. was stirred at room temp. for 1·5 hr. The soln. was acidified with 0·2 N HCl, stirred 2 min, then made basic with Na_2CO_3 and extracted with $CHCl_3$; yield, 35 mg, m.p. 132–135°, $[\alpha]_D^{26°}$ −181° (*c* 0·44, $CHCl_3$). TLC showed only one spot, which was identical to natural cephalotaxine by all our criteria.

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Note added in proof: A recent publication incorporates portions of McKay's Thesis[2]; PAUDLER, W. W. and MCKAY, J. (1973) *J. Org. Chem.* **38**, 2110.