Phytochemistry, 1973, Vol. 12, pp. 2521 to 2525. Pergamon Press. Printed in England.

MINOR INDOLOPYRIDOQUINAZOLINE ALKALOIDS FROM *EUXYLOPHORA PARAËNSIS*

BRUNO DANIELI, GIOVANNI PALMISANO and GIOVANNI RUSSO

Istituto di Chimica Organica della Facoltà di Scienze, Università degli Studi di Milano, via C. Saldini 50, 20133 Milano, Italia

and

GIORGIO FERRARI

Laboratori Ricerche Simes, Milano, Italia

(*Received* 19 *December* 1972. *Accepted* 25 *April* 1973

Key Word Index—*Euxylophora paraënsis*; Rutaceae; alkaloids; indolopyridoquinazolines; euxylophorines-*C* and -*D*; euxylophoricines-*D* and -*E*.

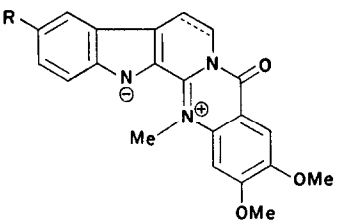
Abstract—Four new indolopyridoquinazoline alkaloids were isolated in small amount from the bark of *Euxylophora paraënsis* Hub. Chemical reactions and spectroscopic evidence indicated that euxylophorine-*C* has structure (Ia), which was confirmed by synthesis. The structures of the other alkaloids, euxylophorine-*D* (IIa), euxylophoricine-*D* (IIIa) and euxylophoricine-*E* (IVa), were determined through correlation with (Ia).

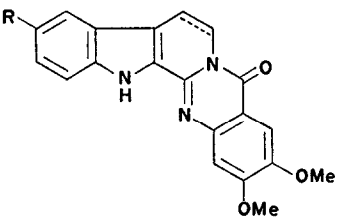
INTRODUCTION

IN PREVIOUS papers,[1-3] we reported the structure determination of six indolopyridoquinazoline alkaloids isolated from the bark of *Euxylophora paraënsis* Hub. (Rutaceae). The present communication relates to the identification of four new alkaloids of the same type (Ia), (IIa), (IIIa) and (IVa), which we have named euxylophorine-*C*, euxylophorine-*D*, euxylophoricine-*D* and euxylophoricine-*E* respectively.

RESULTS AND DISCUSSION

The new alkaloids are present in small amount and are structurally related to the already known euxylophorine-*A* (Ib),[1] euxylophorine-*B* (IIb),[2] euxylophoricine-*A* (IIIb)[1] and euxylophoricine-*B* (IVb).[1] Although the new alkaloids have a TLC behaviour very similar to the above-mentioned compounds, they are easily distinguishable by their different fluorescence at 350 nm.





(Ia)	R = OMe		(IIIa)	R = OMe	
(IIa)	R = OMe	double bond at 7, 8	(IVa)	R = OMe	double bond at 7, 8
(Ib)	R = H		(IIIb)	R = H	
(IIb)	R = H	double bond at 7, 8	(IVb)	R = H	double bond at 7, 8

[1] CANONICA, L., DANIELI, B., MANITTO, P., RUSSO, G. and FERRARI, G. (1968) *Tetrahedron Letters* 4865.
[2] DANIELI, B., MANITTO, P., RONCHETTI, F., RUSSO, G. and FERRARI, G. (1972) *Experientia* **28**, 249.
[3] DANIELI, B., MANITTO, P., RONCHETTI, F., RUSSO, G. and FERRARI, G. (1972) *Phytochemistry* **11**, 1833.

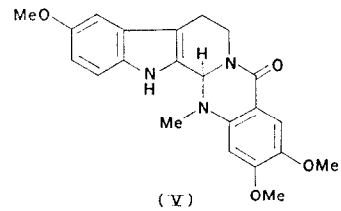
The new bases were isolated by alumina chromatography of the methaonlic extract of the bark.

Euxylophorine-*C* (Ia), $C_{22}H_{21}N_3O_4$, M^+ at *m/e* 391, crystallized from anhydrous benzene as red needles, m.p. 207–209° (dec.). Its IR spectrum in Nujol exhibited a carbonyl peak at 1665 cm^{-1} and unsaturation bands at 1620 and 1555 cm^{-1}. The UV spectrum in $CHCl_3$ showed a maximum at 425 nm (log ϵ 4·51), which shifts to 408 nm in anhyd. CH_3CN and to 389 nm in absolute EtOH.

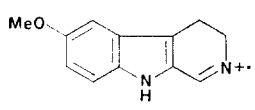
Owing to the poor solubility in $CDCl_3$ and pyridine-d_5, the NMR spectrum was carried out in CF_3COOH containing 20% of $CDCl_3$ and it corresponded to the trifluoracetate of (Ia). The spectrum exhibited two triplets at δ 3·40 and 4·75 (*J* 7 Hz) for the $\geqslant C-CH_2-CH_2-N \leqslant$ sequence, three singlets at δ 4·02, 4·12 and 4·15 for the –OMe groups, a broad singlet at δ 4·50 ($W_{\frac{1}{2}} = 3$ Hz) for the $\geqslant N_{14}-CH_3$, four aromatic protons between δ 7·2 and 7·6, an aromatic proton as a singlet at δ 7·85 and a NH proton at δ 10·1.

The addition of a large quantity of benzene to trifluoracetic solution of the NMR, gave green–yellow crystals of the trifluoracetate of (Ia), m.p. 194–198° (dec.), which could be converted into the free base on treatment with aqueuos ammonia, followed by chloroform extraction.

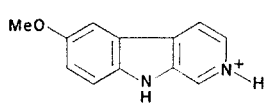
When a methanolic solution of (Ia) was reduced with $NaBH_4$, the dihydro derivative (V), $C_{22}H_{23}N_3O_4$, M^+ at *m/e* 393, m.p. 244–248° (dec.) from MeOH–$CHCl_3$, λ_{max} (CH_3CN) 268 nm (log ϵ 4·18), was obtained.



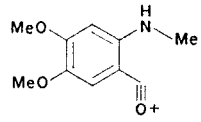
(V)



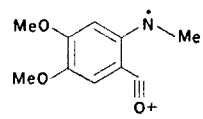
(a)



(b)



(c)



(d)

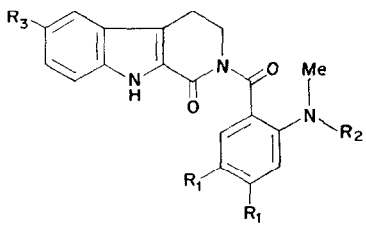
Whereas (Ia) shows a very poor fragmentation, (V) exhibits conspicuous and diagnostically valuable MS peaks at *m/e* 200 (14%), 199 (22%), 194 (91%) and 193 (38%). By analogy with related compounds,[4] structures (*a*), (*b*), (*c*) and (*d*) respectively are attributed to these ions.

The presence of ions (*c*), (*d*) and the signal for one aromatic proton as a singlet at δ 7·85 in the NMR spectrum of (Ia) due to C–1*H*, allowed to locate two–OMe groups on ring E at C-2 and C-3. The third –OMe group could only be assigned to ring A and its position was determined from the UV spectrum of the hydrated form (VIa) of (Ia).

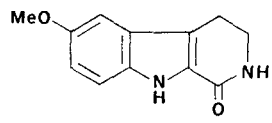
When a sample of (Ia) was dissolved in boiling benzene and allowed to stand without exclusion of moisture or when water is added to a pyridine solution, a pale yellow solid separated. This compound turned red upon heating and had the same m.p. as (Ia). Elemental analysis indicated the molecular formula $C_{22}H_{23}N_3O_5$ resulting from the

[4] BUDZIKIEWICZ, H., DJERASSI, C. and WILLIAMS, D. H. (1964) *Structure Elucidations of Natural Products by Mass Spectrometry*, Vol. I, 80, Holden Day, San Francisco.

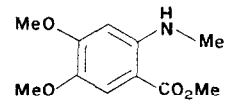
addition of a molecule of water to (Ia). The IR spectrum in Nujol showed two NH bands at 3360 and 3260 cm^{-1} as well as two carbonyl bands at 1660 and 1650 cm^{-1}, in agreement with the structure (VIa) of 2-[4,5-dimethoxy-2-methylaminobenzoyl]-6-methoxy-1,2,3,4-tetrahydronorharmanone-1. The UV spectrum of (VIa) in anhyd. CH_3CN showed two maxima at 318 and 395 nm.* The maximum at 318 nm is characteristic of the form (VIa): it is the same as that of the model compound (VII)[5] and shifts to a longer wavelength to 308 nm, the max. of the hydrated form (VIb) of (Ib) obtained in a similar way through crystallization of (Ib) from benzene.



(VI a) R_1, R_3 = OMe; R_2 = H
(VI b) R_1 = OMe; R_2, R_3 = H
(VII) R_1 = H, R_2 = Me; R_3 = OMe



(VIII)



(IX)

On the other hand, the dihydro derivative (V) shows a UV maximum which confirms the position of this methoxyl group.

Conclusive proof for the structure (Ia) of euxylophorine-*C* was obtained through synthesis from 6-methoxy-1,2,3,4-tetrahydronorharmanone-1 (VIII) and methyl 4,5-dimethoxy-2-methylaminobenzoate (IX) in boiling toluene in presence of $POCl_3$.[6] The red product thus obtained was identical in all respects to the natural euxylophorine-*C*.

Euxylophorine-*D* (IIa), $C_{22}H_{19}N_3O_4$, M^+ at *m/e* 389, crystallized from benzene in yellow–orange needles, m.p. 256–260° (dec.). Its UV spectrum in CH_3CN showed maxima at 277 and 360 nm (log ϵ 4·33 and 4·65) and was very similar to the one of (IIb).[2] On the basis of this analogy and of the co-occurrence with (Ia), structure (IIa) was proposed for euxylophorine-*D* This was confirmed by synthesis through dehydrogenation of (Ia) with DDQ in boiling benzene

The third alkaloid, euxylophoricine-*D* (IIIa), $C_{21}H_{19}N_3O_4$, M^+ at *m/e* 377, is a white solid which crystallized from $CHCl_3$, m p. 293–295°. The IR and UV spectra were very similar to these of euxylophoricine-*A* (IIIb),[1] as well as the NMR spectrum in CF_3COOH + 20% $CDCl_3$ which showed two symmetrical triplets at δ 3·43 and 4·78 (*J* 7 Hz) for the $\geqslant C-CH_2-CH_2-N \leqslant$ sequence, two singlets at δ 4·03 and 4·10 for the three –OMe groups, four aromatic protons between δ 7·2 and 7·6, an aromatic proton as a singlet at δ 7·78 and a NH proton at δ 7·95. By analogy with the above compounds, it was thought that the three methoxy groups were at the same position as in (Ia) and (IIa). That this was the case was demonstrated through the pyrolysis of the trifluoracetate of euxylophorine-*C* which gave pure euxylophoricine-*D* identical in all respects with the natural compound.

* It is not possible to calculate the intensity of these maxima because in this solvent equilibration between the forms (VIa) and (Ia) takes place: the UV spectrum changes showing a slight decrease in intensity of the first maximum, a shift towards 408 nm and a large increase of the second maximum.

[5] PACHTER, I. J., MOHRBACHER, R. J. and ZACHARIAS, D. E. (1961) *J. Am. Chem. Soc.* **83**, 638.
[6] PACHTER, I. J., RAFFAUF, R. F., ULLYOT, G. E. and RIBEIRO, O. (1960) *J. Am. Chem. Soc.* **82**, 5187.

Euxylophoricine-*E* (IVa), $C_{21}H_{17}N_3O_4$, is a yellow solid which crystallized from $CHCl_3$ and gave strong fluorescent solutions. Its structure was deduced from the UV maxima in $CHCl_3$ at 255, 289, 292 (sh), 307, 355, 376 and 398 nm (log ϵ 4·65, 4·55, 4·48, 4·54, 4·57, 4·62 and 4·64) very similar to these of (IVb)[1] and confirmed by dehydrogenation of (IIIa) with selenium at 300°.

EXPERIMENTAL

Capillary m.ps were uncorrected. The spectra were determined as follows: NMR at 60 MHz in CF_3COOH containing 20% $CDCl_3$ with TMS as internal standard; IR in Nujol; MS on an LKB 9000 equipped with DIS. Alumina Woelm (activity III) was used for column chromatography and TLC were performed on DC Fertigplatten Kieselgel F_{254} Merck, the usual solvent being AcOET–toluene–HCOOH, 4:5:1. Spots were visualized in UV light at 350 nm.

Extraction and isolation. 3·5 kg bark of *Euxylophora paraënsis* Hub. was extracted with MeOH and euxylophorine-*A* was isolated as previously described.[3] The residue of the mother liquors of the crystallization of euxylophorine-*A* was chromatographed on 50 g of alumina and eluted sequentially with C_6H_6, C_6H_6–acetone (up to 15% of acetone) and then with C_6H_6–acetone–$NHEt_2$, 85:15:1.

Euxylophoricine-D (IIIa). Elution with C_6H_6 gave, after pure euxylophoricine-*A* (IIIb),[1] fractions containing the above mentioned compound and a new slightly more polar compound having a different fluorescence. The residue of the pooled fractions was rechromatographed on alumina and gave a new product (R_f 0·67) with a strong yellow fluorescent spot in TLC. Crystallization from $CHCl_3$ gave 17 mg euxylophoricine-*D* as plates, m.p. 293–295°, M^+ 377 (base peak); ν_{max} 3450, 1670, 1620 and 1600 cm^{-1}; λ_{max} (CH_3CN) 252, 341, 356 and 374 nm (log ϵ 4·44, 4·45, 4·48 and 4·32); NMR: 3·43 δ (2H, *t*, *J* 7 Hz, C-8H_2), 4·03 (3H, *s*, –OMe), 4·10 (6H, *s*, –OMe), 4·78 (2H, *t*, *J* 7 Hz, C-7H_2), 7·2–7·6 (4H, *m*, aromatic protons), 7·78 (1H, *s*, C-4*H*) and 7·95 (1H, *s br*, N-*H*).

Euxylophoricine-E (IVa). Elution with C_6H_6–acetone (99:1) gave euxylophoricine-*B* (IVb) and a mixture of this alkaloid with a new compound which could be isolated after repeated chromatography. Euxylophoricine-*E* showed pale yellow fluorescent spot with R_f 0·62 and for crystallization from $CHCl_3$ 10 mg of yellowish crystals was obtained, m.p. 290°; M^+ 375 (base peak); λ_{max} ($CHCl_3$) 255, 289, 292 (sh), 307, 355, 376 and 398 nm (log ϵ 4·65, 4·55, 4·48, 4·54, 4·57, 4·62 and 4·64).

Synthesis of euxylophoricine-D. 20 mg of euxylophorine-*C* trifluoroacetate was heated at 260° for 1 hr. The residue was chromatographed on alumina eluting with benzene: 8 mg of pure euxylophoricine-*D* was obtained, identical in all respects to the natural material.

Conversion of euxylophoricine-D *into euxylophoricine*-E. 10 mg of euxylophoricine-*D* was mixed with an excess of selenium powder and heated at 300° for 10 hr. Extraction with MeOH and crystallization from $CHCl_3$ gave 7 mg of a product identical to the natural euxylophoricine-*E*.

Euxylophorine-C (Ia) *and euxylophorine*-D (IIa). The C_6H_6–acetone–$NHEt_2$ (85:15:1) eluates contained sequentially euxylophorine-*A*, a mixture of this alkaloid and euxylophorine-*C*, pure euxylophorine-*B* and a mixture of the latter and euxylophorine-*D*. The new compounds could be isolated only through chromatography of the corresponding fractions eluting with AcOEt containing increasing concentration of $NHEt_2$ up to 1%. Euxylophorine-*C* (R_f 0·15, pale yellow fluorescent spot in TLC) crystallized from anhydrous benzene in red needles, m.p. 207–209° (dec.). (Found: C, 66·91; H, 5·28; N, 10·80. $C_{22}H_{21}N_3O_4$ required: C, 67·51; H, 5·41; N, 10·74%), M^+391; ν_{max} 1665, 1620 and 1555 cm^{-1}; λ_{max} (CH_3CN) 303 and 408 nm (log ϵ 4·14 and 4·40); NMR: 3·40 δ (2H, *t*, *J* 7 Hz, C-8H_2), 4·02 (3H, *s*, –OMe), 4·12 (3H, *s*, –OMe), 4·15 (3H, *s*, –OMe), 4·50 (3H, *s br*, $W_{\frac{1}{2}}$ = 3 Hz, N_{14}–Me), 4·75 (2H, *t*, *J* 7 Hz, C-7H_2), 7·2–7·6 (4H, *m*, aromatic protons), 7·85 (1H, *s*, C-4*H*) and 10·1 (1H, *s br*, N_{13}-H). Euxylophorine-*D* (R_f 0·11, yellow fluorescent spot in TLC) crystallized from anhyd. C_6H_6 or $CHCl_3$–isopropyl ether as yellow–orange needles, m.p. 256–260° (dec.), M^+ 389; λ_{max} (CH_3CN) 277 and 360 nm (log ϵ 4·33 and 4·65).

Synthesis of euxylophorine-C. To a solution of 50 mg of 6-methoxy-1,2,3,4-tetrahydronorharmanone-1 in 30 ml of dry boiling toluene, 0·03 ml of freshly distilled $POCl_3$ was added. The reaction mixture was stirred for 30 min and then 100 mg of methyl 4,5-dimethoxy-2-methylamino benzoate was added. Heating was continued under reflux for 3 hr. The toluene layer was decanted and the deep yellow precipitate was treated with aq. NH_3 and $CHCl_3$. The red $CHCl_3$ solution was dried and evaporated. After crystallization from anhyd. C_6H_6, 43 mg of pure euxylophorine-*C* was obtained, identical in TLC, m.p., UV, IR with the natural product.

Conversion of euxylophorine-C *into euxylophorine*-D. 10 mg of euxylophorine-*C* in 20 ml of anhyd. C_6H_6 was treated with 12 mg of DDQ in 10 ml C_6H_6. The solution was refluxed for 2 hr, the solvent was evaporated and the residue chromatographed on alumina. Eluting with AcOEt–$NHEt_2$ (99:1) gave 3 mg of a product identical in all respects to the natural one.

Reduction of (Ia) *into the dihydro derivative* (V). To a stirred solution of 15 mg of euxylophorine-*C* in 25 ml of MeOH, 15 mg of $NaBH_4$ was added in small portions at r.t. The stirring had been continued for 1 hr, the

solvent was evaporated and the residue additioned with H_2O and repeatedly extracted with $CHCl_3$. Removal of solvent left a crystalline solid, which was crystallized from MeOH–$CHCl_3$, m.p. 244–248°; M^+ 393; λ_{max} (CH_3CN) 268 nm (log ϵ 4·18).

Trifluoracetate of euxylophorine-C. 20 mg euxylophorine-*C* was dissolved in 0·5 ml trifluoracetic acid; for dilution with C_6H_6 a crystalline green–yellow solid was obtained, m.p. 194–198° (dec.); λ_{max} ($CHCl_3$) 247, 307 and 400 nm (log ϵ 4·31, 4·14 and 4·33).

2-[4,5-*dimethoxy*-2-*methylaminobenzoyl*]-6-*methoxy*-1,2,3,4-*tetrahydronorharmanone*-1 (VIa). 5 mg of euxylophorine-*C* was dissolved in 10 ml of boiling C_6H_6 and allowed to stand at r.t. A pale yellow solid separated which showed the same m.p. as (Ia). (Found: C, 64·09; H, 5·87; N, 10·78. $C_{22}H_{23}N_3O_5$ required: C, 64·54; H, 5·66; N, 10·26%). ν_{max} 3360, 3260, 1660 and 1650 cm^{-1}; λ_{max} (CH_3CN) 318 and 395 nm.

Acknowledgements—The authors thank Professor L. Canonica for his kind advice, Dr. G. Severini Ricca for the NMR spectra and Dr. T. Salvatori for the MS spectra.