Phytochemistry, 1974, Vol 13, pp 1603 to 1606 Pergamon Press Printed

1-HYDROXYRUTAECARPINE FROM *EUXYLOPHORA PARAËNSIS*

BRUNO DANIELI, GIOVANNI PALMISANO, GUGLIELMO RAINOLDI and GIOVANNI RUSSO

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

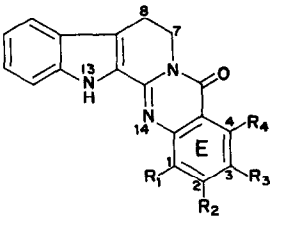
(*Received* 19 *October* 1973)

Key Word Index—*Euxylophora paraensis*, Rutaceae, alkaloids; indolopyridoquinazolines, 1-hydroxyrutaecarpine

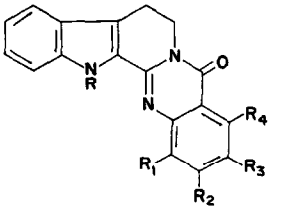
Abstract—The structure of the title compound has been determined on the basis of spectroscopic evidence and synthesis

INTRODUCTION

CONTINUING our studies[1-4] on the constituents of the bark of *Euxylophora paraënsis* Hub. (Rutaceae), we report now the isolation and characterization of a further new indolopyridoquinazoline alkaloid. The structure was established as 1-hydroxy-8,13-dihydro[2′,3′-3,4] pyrido[2,3-b]quinazolin-5(7H)-one (**1a**) or briefly 1-hydroxyrutaecarpine, on the basis of spectroscopic and chemical evidence.



(**2**) R = H



(**3**) R = Me

(1a) R_1 = OH ; $R_2 = R_3 = R_4$ = H
(1b) R_2 = OH ; $R_1 = R_3 = R_4$ = H
(1c) R_3 = OH ; $R_1 = R_2 = R_4$ = H
(1d) R_4 = OH ; $R_1 = R_2 = R_3$ = H

(a) R_1 = OMe; $R_2 = R_3 = R_4$ = H
(b) R_2 = OMe; $R_1 = R_3 = R_4$ = H
(c) R_3 = OMe; $R_1 = R_2 = R_4$ = H
(d) R_4 = OMe; $R_1 = R_2 = R_3$ = H
(e) $R_1 = R_2 = R_3 = R_4$ = H

RESULTS AND DISCUSSION

The new alkaloid was eluted by MeOH from an alumina column after elution of the other alkaloids and was completely purified through repeated silica gel chromatography. Crystallization from $CHCl_3$–MeOH afforded pure (**1a**) as pale yellow needles, m.p. 318–320°. Elemental analysis and MS (M^+ at *m/e* 303) indicated a molecular formula $C_{18}H_{13}N_3O_2$.

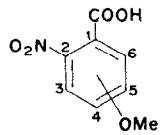
[1] CANONICA, L., DANIELI, B., MANITTO, P., RUSSO, G and FERRARI, G (1968) *Tetrahedron Letters* 4865
[2] DANIELI, B., MANITTO, P., RONCHETTI, F., RUSSO, G. and FERRARI, G (1972) *Experientia* **28**, 249.
[3] DANIELI, B., MANITTO, P., RONCHETTI, F., RUSSO, G and FERRARI, G (1972) *Phytochemistry* **11**, 1833
[4] DANIELI, B., PALMISANO, G RUSSO, G and FERRARI, G (1973) *Phytochemistry* **12**, 2521

The spectral data are strongly suggestive that (**1a**) is a hydroxy-derivative of rutaecarpine (**2e**), $C_{18}H_{13}N_3O$.[5] A bathochromic shift in the presence of a base evidenced the phenolic nature of the OH group.

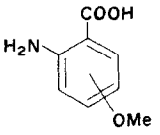
The MS of (**1a**) showed a poor fragmentation. In addition to the molecular ion at *m/e* 303, low intensity ions at *m/e* 169, 168, 167, 155, 115 can be attributed to the carbolinic portion of the molecule [6] The same fragments are present in rutaecarpine, indicating that the carbolinic system is not substituted and the OH group is located on ring E

The NMR spectrum in DMSO-d_6 exhibits two triplets at δ 3·18 and 4·43 (J 7Hz) for the $\geqslant C-CH_2-CH_2-N<$ sequence, two broad singlets at δ 9·20 and 11·49 for O<u>H</u> and N<u>H</u> respectively and a complex multiplet between δ 7–7·8 for seven aromatic protons As the splitting pattern of the aromatic region is too complicated to analyse, we could not obtain any information about the exact location of the OH group and it was decided to synthesize the 4 possible hydroxyrutaecarpines (**1a,b,c,d**) For experimental reasons, we thought that a better comparison could be effected on the corresponding methylether derivatives (**2a,b,c,d**)

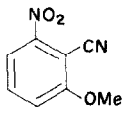
A simple and almost quantitative methylation of (**1a**) was achieved by refluxing with MeI K_2CO_3 in anhydrous acetone. Surprisingly during the course of the reaction both the phenolic hydroxyl and the indolic nitrogen N_{13} are methylated[7] to afford (**3a**), $C_{20}H_{17}N_3O_2$, m p 187–189° (from C_6H_6). Therefore, the comparison was made with this compound.



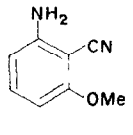
(**4 a**) 3-methoxy
(**4 b**) 4-methoxy
(**4 c**) 5-methoxy
6-methoxy



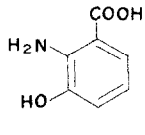
(**5 a**)
(**5 b**)
(**5 c**)
(**5 d**)



(**6**)



(**7**)



(**8**)

Compounds (**2a,b,c,d**) were synthesized by condensation of 1,2,3,4-tetrahydronorharman-1-one with the appropriate anthranilic acids in the presence of $POCl_3$.[8] The isomeric 4- and 5-methoxyanthranilic acids (**5b,c**) were prepared from the corresponding nitro acids (**4b,c**) using standard procedures

The fourth isomer 6-methoxyanthranilic acid (**5d**) was obtained from 2-methoxy-6-nitrobenzonitrile (**6**) by catalytic reduction to (**7**) and subsequent hydrolysis

Treatment of (**2a,b,c,d**) under the aforementioned methylation condition yielded the corresponding N_{13}-methyl derivatives (**3a,b,c,d**) A comparison of the physico-chemical data (see Experimental) and, in particular, of the aromatic regions of the NMR spectra in DMSO-d_6 left no doubt as to the identity of (**3a**) with the product obtained from the natural compound. Therefore the OH group must be located at position **1** as shown in (**1a**).

Finally, synthetic 1-hydroxyrutaecarpine, obtained from 3-hydroxyanthranilic acid (**8**) and 1,2,3,4-tetrahydronorharman-1-one, was identical in spectral and chromatographic comparisons with the natural product

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[6] BUDZIKIEWICZ, H, DJERASSI, C and WILLIAMS, D H (1964) *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol I, Holden Day, San Francisco
[7] DANIELI, B and PALMISANO, G *Gazz Chim Ital* Submitted
[8] LORENZ, R (1958) *Ger Pat* 1,039,065

EXPERIMENTAL

All capillary m p 's are uncorrected. The spectra were determined as follows: NMR in DMSO-d_6 with TMS as internal standard if not otherwise stated, UV in MeOH, MS on a LKB 9000 equipped with DIS Column chromatographic separations were effected with alumina Woelm (activity **3**) and silica gel TLC was performed on DC Fertigplatten Kieselgel F_{254} (Merck) the usual solvent being $CHCl_3$–EtOAc (8 2) Spots were visualized in UV light at 350 nm

Extraction and isolation After elution of euxylophorine C and D^4 with C_6H_6–Me_2CO–$NHEt_2$ (85 15·1) the alumina was washed with MeOH The residue was rechromatographed on silica gel eluting with C_6H_6–EtOH (98 2) The fractions containing a main pale yellow fluorescent spot with R_f 0 29 were pooled and chromatographed under the same conditions to afford 63 mg of crude (**1**). Crystallization from $CHCl_3$–MeOH gave pure 1-hydroxyrutaecarpine as pale yellow needles, m p 318–320° (Found C, 70·91, H, 4 23; N, 13·62 $C_{18}H_{13}N_3O_2$ required C, 71 28, H, 4 32, N, 13 85%, MS *m/e* 303 (M^+, 100%), 302 (75%), 169 (4 5%), 168 (5 4%), 167 (5 8%), 155 (6 6%), 115 (6 2%), ν_{max} (Nujol) 3320, 3280, 1645, 1622, 1603 cm^{-1}, λ_{max} (MeOH) 287, 297, 332, 348, 361 and 379 nm (log ϵ 3 80, 3·86, 4 37, 4 41, 4 51 and 4 40), λ_{max} (MeOH + 1 N NaOH) 309 and 393 nm, NMR δ 3 18 (2H, *t*, *J* 7Hz, C-8$\underline{H}_2$), 4 43 (2H, *t*, *J* 7 Hz, C-7$\underline{H}_2$), 7 0–7 8 (7 H, complex *m*, aromatic protons), 9 20 (1 H, broad *s*, OH), 11·49 (1H, broad *s*, N_{13}-$\underline{H}$); NMR ($\overline{CF_3}COOH$ + 20% $CDCl_3$) δ 3 46 (2 H, *t*, *J* 7 Hz, C-8$\underline{H}_2$), 4 80 (2H, *t*, *J* 7 Hz, C-7$\underline{H}_2$)

1-*Hydroxyrutaecarpine hydrochloride* (**1a**. HCl). Yellow needles, m p 326–328° (from MeOH–$CHCl_3$) (Found C, 63 84, H, 4 09, N, 12 48 $C_{18}H_{14}ClN_3O_2$ required· C, 63·64, H, 4 12, N, 12 36%)

Synthesis of 1-*hydroxyrutaecarpine* (**1a**) 2 ml freshly dist. $POCl_3$ was added to 56 mg of 1,2,3,4-tetrahydronorharman-1-one and kept at 60° for 2 min Addition of 5 ml dry Et_2O to the cold clear soln deposited a sticky lemon-coloured solid which was collected by decantation and washed well with dry Et_2O The solid was quickly dissolved in 10 ml of dry C_6H_6 with 46 mg of 3-hydroxyanthranilic acid (**8**) and refluxed for 3 hr The C_6H_6 layer was decanted and the brown–yellow ppt treated with 20% Na_2CO_3 soln and repeatedly extracted with $CHCl_3$ The combined extracts were washed with H_2O, dried ($MgSO_4$) and evaporated The residue was crystallized from $CHCl_3$–MeOH to afford 67 mg (74%) of pure 1-hydroxyrutaecarpine (**1**), identical in all respects to the natural product

1-*methoxyrutaecarpine* (**2a**) Yellow plates, m p 232–234° (from C_6H_6-hexane); R_f 0 44, violet fluorescent spot

2-*Methoxyrutaecarpine* (**2b**) Yellow needles, m p 253°, R_f 0 38, violet fluorescent spot, λ_{max} 332, 343, 360 nm (log ϵ 4 43, 4 69, 4 40), NMR δ 3 14 (2H, *t*, *J* 7 Hz, C-8$\underline{H}_2$), 3 90 (3H, *s*, O-$\underline{Me}$), 4 42 (2H, *t*, *J* 7 Hz, C-7$\underline{H}_2$), 7–7 7 (6H, complex *m*, aromatic protons), 8 05 (1H, *d*, *J* 9 Hz, C-4$\underline{H}$), 11 79 (1H, broad *s*, $\underline{NH}$)

3-*Methoxyrutaecarpine* (**2c**) Pale yellow needles, m p 278–281° (from C_6H_6–$CH\underline{Cl}_3$), R_f 0 39, violet fluorescent spot, λ_{max} 339, 348, 367 nm (log ϵ 4 49, 4 48, 4 32), NMR δ 3 19 (2H, *t*, *J* 7 Hz, C-8$\underline{H}_2$), 3 91 (3H, *s*, O$\underline{Me}$), 4 49 (2H, *t*, *J* 7 Hz, C-7$\underline{H}_2$), 7–7 8 (7 H, complex *m*, aromatic protons), 12 8 (1H, broad *s*, $\underline{NH}$)

4-*Methoxyrutaecarpine* (**2d**), Needles, m p 320° (from $CHCl_3$–C_6H_6), R_f 0 25, violet fluorescent spot, NMR δ 3 14 (2H, *t*, *J* 7 Hz, C-8$\underline{H}_2$), 3 88 (3H, *s*, O-$\underline{Me}$), 4 35 (2H, *t*, *J* 7 Hz, C-7$\underline{H}_2$) 6 9–7 8 (7H, complex *m*, aromatic protons), 11 75 (1H, broad *s*, N–$\underline{H}$)

Conversion of (**1a**) *into* N_{13}-*methyl*-1-*methoxyrutaecarpine* (**3a**) 1 ml MeI and 40 mg fused K_2CO_3 were added to a soln of 20 mg of (**1a**) in 50 ml of dry acetone The reaction mixture was refluxed and monitored by TLC. After 12 hr the reaction was complete and then filtered to remove solid K_2CO_3 The solvent was evaporated *in vacuo*, the residue washed with H_2O and extracted with $CHCl_3$ Evaporation gave 19 mg of a yellow solid which was crystallized from C_6H_6 to furnish pure (**3a**) as pale yellow needles, m p 187–189° (Found C, 72·22, H, 5 11, N, 12 58 $C_{20}H_{17}N_3O_2$ required C, 72 49, H, 5 17, N, 12·68%), R_f 0 54, dark blue fluorescent spot, ν_{max} ($CHCl_3$) 1670, 1610, 1592 cm^{-1}, λ_{max} 283, 295, 332, 346, 362 and 380 nm (log ϵ 3 80, 3 86, 4 47, 4·53, 4 62 and 4 52), NMR δ 3 14 (2H, *t*, *J* 7 Hz, C-8$\underline{H}_2$), 3 95 (3H, *s*, O-$\underline{Me}$), 4 32 (3H, *s*, N-$\underline{Me}$), 4 42 (2H, *t*, *J* 7 Hz, C-7$\underline{H}_2$), 7 07–7 77 (7H, complex *m*, aromatic protons)

N_{13}-*methyl*-2-*methoxyrutaecarpine* (**3b**) Plates, m p 164° (from EtOAc), R_f 0 55, brilliant dark blue fluorescent spot, ν_{max} (Nujol) 1662 cm^{-1}, λ_{max} 334, 346, 362 nm (log ϵ 4 44, 4 49, 4 40), NMR δ 3 12 (2H, *t*, *J* 7 Hz, C-8$\underline{H}_2$), 3 89 (3H, *s*, O-$\underline{Me}$), 4 26 (3H, *s*, N-$\underline{Me}$), 4 38 (2H, *t*, *J* 7 Hz, C-7$\underline{H}_2$), 6 9–7 7 (6H, complex *m*, aromatic protons) 8·05 (1H, *d*, *J* 8 Hz, C-4$\underline{H}$)

N_{13}-*methyl*-3-*methoxyrutaecarpine* (**3c**) Needles, m p 185° (from $CHCl_3$–hexane), R_f 0 50, dark blue fluorescent spot, ν_{max} (Nujol) 1663, 1618, 1592 cm^{-1}, λ_{max} 341, 353, 373 nm (log ϵ 4 32, 4 22, 4 13), NMR δ 3 15 (2H, *t*, *J* 7 Hz, C-8$\underline{H}_2$), 3 89 (3H, *s*, O-$\underline{Me}$), 4·28 (3H, *s*, N-$\underline{Me}$), 4·46 (2H, *t*, *J* 7 Hz, C-7$\underline{H}_2$), 7 1–7 9 (7H, complex *m*, aromatic protons)

N_{13}-*methyl*-4-*methoxyrutaecarpine* (**3d**) Needles, m p. 224–226° (from EtOAc), R_f 0 39, dark blue fluorescent spot, ν_{max} (Nujol) 1675 cm^{-1}, NMR δ 3 15 (2H, *t*, *J* 7 Hz, C-8$\underline{H}_2$), 3 85 (3H, *s*, O-$\underline{Me}$), 4·23 (3H, *s*, N-$\underline{Me}$), 4 33 (2H, *t*, *J* 7 Hz, C-7$\underline{H}_2$), 6 9–7 9 (7H, complex *m*, aromatic protons)

4-*Methoxy*-2-*nitrobenzoic acid* (**4b**) Leaflets, m p 194° (lit[9] 195–196°), ν_{max} (Nujol) 1705 cm^{-1}, NMR δ 3 87 (3H, *s*, O-$\underline{Me}$), 7 25 (1H, *dd*, J_1 9 Hz, J_2 2 5 Hz, C-5H), 7 45 (1H, *d*, *J* 2 5 Hz, C-3$\underline{H}$), 7 85 (1H, *d*, *J* 9 Hz, C-6$\underline{H}$)

[9] ULLMANN, F and DOOTSON, P (1918) *Chem Ber* **51**, 20

5-*Methoxy-2-nitrobenzoic acid* (**4c**). Pale yellow needles, m p 133° (lit[10] 130–131°), ν_{max} (Nujol) 1695, 1720 cm^{-1}, NMR δ 3·88 (3H, *s*, O-Me), 7·18 (1H, *dd*, J_1 10 Hz, J_2 2·5 Hz, C-4H), 7·20 (1H, *J* 2·5 Hz C-6H) 8·05 (1H, *d*, *J* 10 Hz, C-3H)

4-*Methoxyanthranilic acid* (**5b**) Needles, m p 172° (from H_2O) (lit[9] 172°), ν_{max} (Nujol) 3460, 3360, 1670 cm^{-1} NMR δ 3·70 (3H, *s*, O-Me), 6·08 (1H, *dd*, J_1 9 Hz, J_2 3 Hz, C-5H), 6·25 (1H, *d*, *J* 3 Hz, C-3H), 7·61 (1H *d*, *J* 9 Hz, C-6H), 8·20 (2H, broad *s*, NH_2)

5-*Methoxyanthranilic acid* (**5c**) Grey–silver plates, m p 151° (from H_2O) (lit[10] 149°), NMR δ 3·68 (3H, *s*, O-Me), 6·72 (1H, *dd*, J_1 9 Hz, J_2 1 Hz, C-3H), 6·98 (1H, *dd*, J_1 9 Hz, J_2 3·5 Hz, C-4H), 7·25 (1H, *dd*, J_1 3·5 Hz, J_2 1 Hz, C-6H), 7·88 (2H, broad *s*, NH_2)

6-*Methoxyanthranilic acid* (**5d**) 500 mg of 2-amino-6-methoxybenzonitrile (**7**)[12] was dissolved in 50 ml of ethylene glycol in the presence of 20 ml of 30% NaOH soln and refluxed for 36 hr The soln was poured into H_2O, filtered neutralized with dilute HOAc and extracted with Et_2O to yield 485 mg of (**5d**) Crystallization from isopropyl ether–cyclohexane afforded white needles, m p 87° (lit[13] 85–87°), ν_{max} (CCl_4) 1705 cm^{-1}, NMR δ 3·76 (3H, *s*, O-Me), 6·19 (1H, *dd*, J_1 8·5 Hz, J_2 1·5 Hz, C-3H), 6·35 (1H, *dd*, J_1 8·5 Hz, J_2 1·5 Hz, C-5H) 7·09 (1H, *t*, *J* 8·5 Hz, C-4H), 8·04 (2H, broad *s*, NH_2)

2-*Nitro-5-methoxybenzonitrile* (**7**) Amorphous red solid, m p 150–155° (dec) (lit[10] 148–157°), ν_{max} (Nujol) 2230 cm^{-1}, MS *m/e* 148(M^+), 118(M^+-CH_2O), 117(M^+-OCH_3), 105(M^+-CH_3-CO)

2-*Amino-6-methoxybenzonitrile* (**7**) 1·05 g of 2-nitro-6-methoxybenzonitrile (**6**)[11] was dissolved in 150 ml of MeOH and hydrogenated over 210 mg of 10% Pd–C at room temp and atm pres for 6 hr The catalyst was filtered and washed with boiling MeOH The combined fluorescent filtrates were evaporated and re-crystallized from MeOH or EtOAc–hexane to afford 608 mg of pale yellow plates of (**7**), m p 148° (lit[12] 147°), ν_{max} (Nujol) 2220 cm^{-1}, NMR δ 3·80 (3H, *s*, O-Me), 5·12 (2H, broad *s*, NH_2), 6·22 (1H, *dd*, J_1 8 Hz, J_2 2 Hz, C-3H), 6·40 (1 H, *dd*, J_1 8 Hz, J_2 2 Hz, C-5H), 7·20 (1H, *t*, *J* 8 Hz, C-4H)

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

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