

Quinoline Alkaloids. Part XII.¹ Alkaloids and Coumarins of *Orixa japonica* Thunb. Identification of a New Quinoline Alkaloid, Orixinone

By W. J. Donnelly and M. F. Grundon,* The New University of Ulster, Coleraine, Northern Ireland

The coumarin imperatorin and the quinoline alkaloids kokusagine, japonin, and *O*-methylbalfouroninium salt were isolated from the leaves and from the stems of *Orixa japonica* Thunb. A new ketonic quinoline alkaloid, orixinone [2,4-dimethoxy-7,8-methylenedioxy-3-(3-methyl-2-oxobutyl)quinoline], was identified.

INVESTIGATIONS of the alkaloid content of the rutaceous species *Orixa japonica* Thunb have shown that the root bark contains orixine (1),² nor-orixine,² hydroxylun-acridine,³ and five furoquinoline alkaloids;⁴ from the aerial parts of the plant, kokusagine (2),⁴ japonin (3),⁴ and skimmianine⁵ have been obtained.

The diol orixine (1) is of particular interest in connection with our studies of the biosynthesis of 3-isoprenyl-quinoline alkaloids,⁶ and may arise from the corresponding epoxide. Recent work has indicated that the diol evoxine [4; R = CH₂·CH(OH)·C(OH)Me₂] may be

¹ Part XI, T. R. Chamberlain and M. F. Grundon, *J. Chem. Soc. (C)*, 1971, 910.

² M. Terasaka, K. Narahashi, and Y. Tomikawa, *Chem. and Pharm. Bull. (Japan)*, 1960, **8**, 1142.

³ K. Yamamoto and Y. Konno, *Chem. Zentr.*, 1968, **180**, 35.

an artefact derived during the isolation procedure from the related epoxide,⁷ and the possibility that orixine is also an artefact cannot be ruled out. The availability of a small quantity of leaves and stems of *Orixa japonica* prompted us to re-examine the constituents by methods avoiding hydrolysis of any epoxides that might be present. The coumarin and quaternary alkaloid fractions, which have not been studied previously, were also of interest.

The dried leaves and stems of *Orixa japonica* were separately extracted with ethyl acetate, and the extracts

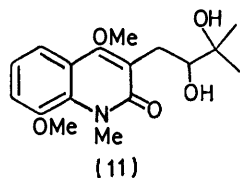
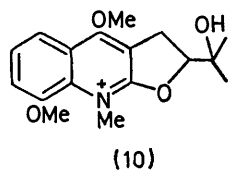
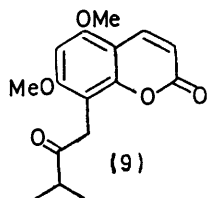
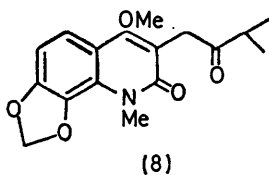
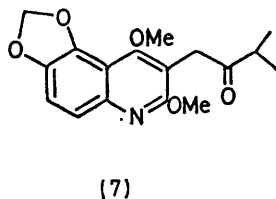
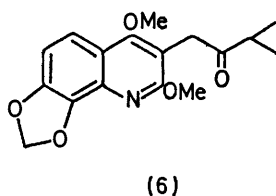
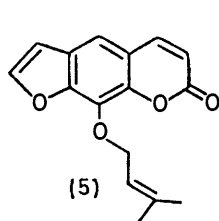
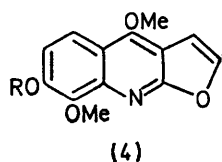
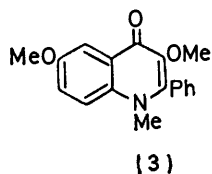
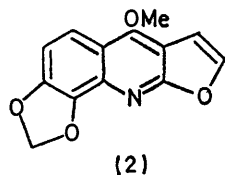
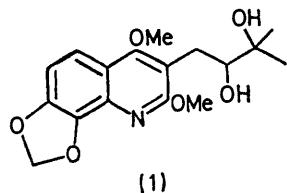
⁴ Ha-hug-Ke, M. Luckner, and J. Reisch, *Phytochemistry*, 1970, **9**, 2199.

⁵ M. Terasaka, *J. Pharm. Soc. Japan*, 1933, **53**, 219.

⁶ R. M. Bowman, J. F. Collins, and M. F. Grundon, *Chem. Comm.*, 1967, 1131.

⁷ D. L. Dreyer, *J. Org. Chem.*, 1970, **35**, 2420.

were chromatographed on alumina. Imperatorin (5) was the only detectable coumarin and was obtained by elution with benzene-chloroform (0.9% in leaves; 0.02% in stems). Further elution gave kokusagine (2) (0.04–0.07%) and japonin (3) (0.06%).



From the stems only there was isolated a new alkaloid (0.025%) for which we propose the name orixinone. The alkaloid, $C_{17}H_{19}NO_5$, was optically inactive and its u.v. spectrum was similar to that of orixine (1).⁸ Its i.r. spectrum lacked hydroxylic absorption but showed

⁸ R. M. Bowman and M. F. Grundon, *J. Chem. Soc. (C)*, 1967, 2368.

⁹ A. Ruegger and D. Stauffacher, *Helv. Chim. Acta*, 1963, **46**, 2329.

a carbonyl peak at 1733 cm^{-1} . Its n.m.r. spectrum resembled that of orixine in the low-field region, showing signals attributable to two *ortho*-coupled aromatic protons and to a methylenedioxy-group. Two methyl resonances were also present. The appearance of a six-proton doublet at $\tau\ 8.84$, a one-proton multiplet at $\tau\ 7.26$, and a two-proton singlet at $\tau\ 6.16$ was consistent with a 3-methyl-2-oxobutyl residue. The presence of this substituent was also indicated by the mass spectrum, which showed a base peak at $(M - 71)^+$ (loss of C_4H_7O). The foregoing evidence is consistent with structure (6) or (7). Orixine (1) was converted into orixinone by treatment with aqueous sulphuric acid at 100° , thus establishing structure (6) for the ketonic alkaloid. Orixinone is apparently not formed from the diol during purification since its isolation was not affected by chromatography on cellulose rather than on alumina.

The ketone (6) and the isomeric lunidonine (8)⁹ are the only examples of quinoline alkaloids containing a 3-methyl-2-oxobutyl group in the 3-position, although this substituent is present in other constituents of rutaceous plants, for example evoxidine (4; $R = CH_2 \cdot CO \cdot CHMe_2$)¹⁰ and the coumarin (9).¹¹ Diols or epoxides may be biosynthetic precursors of the ketones, and might be expected to co-occur. In the present case, however, t.l.c. examination of leaf and of stem extracts of *Orixa japonica* with the synthetic diol (1) and the corresponding epoxide⁸ for comparison showed that the latter compounds were not present in detectable quantities.

The leaves and the stems, after treatment with ethyl acetate, were extracted with water. Quaternary salts were precipitated from the aqueous solution as reineckates, which were converted into chlorides by an ion-exchange method. Both extracts gave the same quaternary alkaloid, isolated as its perchlorate, $[\alpha]_D^{25} +14.8^\circ$, that was shown by comparison with a racemic synthetic sample¹² to be *O*-methylbalfouronium perchlorate (10). The structure was confirmed by alkaline hydrolysis to balfourone (11). *O*-Methylbalfouronium salt, $[\alpha]_D^{25} +9^\circ$, has been obtained previously¹³ from another member of the Rutaceae, *Balfouridendron riedelianum*.

EXPERIMENTAL

N.m.r. spectra were determined with a Perkin-Elmer R12 spectrometer (tetramethylsilane as an internal standard). Optical rotations were measured on a Perkin-Elmer 121 electronic polarimeter.

Isolation of the Constituents.—Plant material was collected in late Autumn from a mature *Orixa japonica* bush at the Botanical Gardens, Dublin. After being dried and ground to a fine powder the leaves (320 g) and stems (385 g) were separately macerated in a blender with ethyl acetate,

¹⁰ F. W. Eastwood, G. H. Hughes, and E. Ritchie, *Austral. J. Chem.*, 1954, **1**, 87.

¹¹ D. L. Dreyer, *Tetrahedron*, 1967, **23**, 4613.

¹² E. A. Clarke and M. F. Grundon, *J. Chem. Soc.*, 1964, 4196.

¹³ H. Rapoport and K. G. Holden, *J. Amer. Chem. Soc.*, 1959, **81**, 3738.

and extraction was continued by stirring with ethyl acetate at 20° for 7 days. Decantation, filtration, and removal of the solvent gave a dark green oily residue, which was chromatographed on deactivated alumina (Woelm, type H). Chromatographic fractions were examined in u.v. light and by spraying with Dragendorff's reagent. Mixed fractions were re-chromatographed. Elution of the leaf extract with benzene-chloroform (4:1) gave imperatorin (5) (290 mg, 0.09%) separating from ether-light petroleum (b.p. 40–60°) in needles, m.p. and mixed m.p. 95–97° (lit.,¹⁴ 95–99°).

Elution with benzene-chloroform (1:4) gave kokusagine (0.07%), m.p. 192–194° (from methanol-ether) (lit.,⁴ 196°), λ_{\max} (EtOH) 251 (ϵ 50,470), τ (CDCl₃) 5.51 (s, OMe), 3.68 (s, CH₂O₂), 2.87 (d, J 2.5 Hz, O·CH=), 2.78 (d, J 9 Hz, ArH), 2.33 (d, J 2.5 Hz, O·C=CH), and 2.02 (d, J 9 Hz, ArH).

Elution with chloroform afforded japonin (200 mg, 0.06%), m.p. 142–143° (from methanol) (lit.,⁴ 143°), λ_{\max} (EtOH) 257 (ϵ 52,480), 347sh, and 355 nm (ϵ 18,100), τ (CDCl₃) 6.45 and 6.32 (each s, NMe and 3-OMe), 6.01 (s, 6-OMe), 2.48–2.80 (7H, m, ArH), and 1.98 (d, J 2.5 Hz, 5-H).

Chromatography of the stem extract in the same manner gave imperatorin (0.025%), kokusagine (0.04%), and japonin (0.06%). Elution with benzene-chloroform (3:2) gave a further compound which was purified by preparative t.l.c. to give a yellow solid (75 mg, 0.025%). This had a u.v. spectrum similar to that of orixine and gave a red colouration with Dragendorff's reagent. Repeated recrystallisation from ether-light petroleum (b.p. 40–60°) gave pale yellow needles of *orixinone* [2,4-dimethoxy-7,8-methylenedioxy-3-(3-methyl-2-oxobutyl)quinoline] (6), m.p. 102–103°, ν_{\max} (CCl₄) 3005, 1733 (CO), 1649, 1624, 1480, 1455, 1408, 1380, 1352, 1278, 1202, 1111, 1065, 1040, and 925 cm⁻¹, τ (CDCl₃) 8.84 (d, J 7.5 Hz, CHMe₂), 7.26 (m, CHMe₂), 6.16 (s, CH₂·CO), 6.10 (s, OMe), 5.96 (s, OMe), 3.81 (s, CH₂O₂), 3.00 (d, J 10 Hz, ArH), and 2.51 (d, J 10 Hz, ArH), m/e 317 (M^+ , 80%), 274 (M^+ - CHMe₂, 2), 246 (M^+ - CO·CHMe₂, 100; m^*), and 231 (M^+ - COCHMe₂ - CH₃) (Found: M^+ , 317.1263. C₁₇H₁₉NO₅ requires M^+ 317.1263).

After extraction with ethyl acetate the plant residue was further extracted with water for 7 days. The aqueous

solution was filtered and treated with a saturated aqueous solution of ammonium reineckate. An immediate white precipitate appeared which was collected by centrifugation. The reineckate salt was passed through a column of Amberlite IRA-400 anion-exchange resin (Cl⁻) in acetone-methanol (1:1). This gave the crude chlorides as a yellow oil (leaves 160 mg, 0.05%; stems 0.10%), which was purified by column chromatography on silicic acid and crystallised with difficulty from methanol-ether to give *O*-methylbalfourodinium chloride as white needles, m.p. 97–100°, $[\alpha]_D^{20} +12.6^\circ$ (in MeOH), λ_{\max} (EtOH) 213 and 253 nm, τ (D₂O) 8.66 and 8.52 (2 × s, Me₂C·OH), 6.02 (m, partially obscured, O·CH·CH₂), 5.92 (s, OMe), 5.63 (s, OMe), 5.44 (s, NMe), 4.69 (m, O·CH·CH₂), and 2.04–2.44 (3H, m, ArH). The chloride was treated with methanolic perchloric acid and the precipitate of *O*-methylbalfourodinium perchlorate was recrystallised from methanol-ether; m.p. 196–198° [lit.,¹³ 124–125° for the (+)-enantiomer; lit.,¹² 203–204° for the racemate], $[\alpha]_D^{20} +14.8^\circ$ (in MeOH) (lit.,¹³ +9°) (Found: C, 50.6; H, 5.5; N, 3.3. Calc. for C₁₇H₂₂ClNO₈: C, 50.6; H, 5.5; N, 3.5%), i.r. spectrum (KBr) virtually identical with that of an authentic specimen of the racemic compound.

(-)-*Balfourolone* (11).—Treatment of *O*-methylbalfourodinium perchlorate with aqueous potassium hydroxide (1.0N) gave (-)-balfourolone almost quantitatively, m.p. 83–86° (from ether-petroleum), $[\alpha]_D^{20} -21.4^\circ$ (methanol) (lit.,¹³ m.p. 99–100° for a sample with $[\alpha]_D^{20} -36^\circ$). This showed an i.r. spectrum (KBr) virtually identical with that of an authentic sample of the racemate.

Synthesis of Orixinone (6).—(±)-Orixine (1) (30 mg; m.p. 148–150°)⁸ was heated in dilute sulphuric acid (20% v/v; 20 ml) at 100° for 45 min. The crude product (26 mg) after neutralisation and extraction with ethyl acetate was purified by preparative t.l.c. to give orixinone (20 mg), m.p. 103–104° [from ether-light petroleum (b.p. 40–60°)], identical (i.r. and mixed m.p.) with the naturally occurring material.

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¹⁴ D. L. Dreyer, *Phytochemistry*, 1969, **8**, 1013.