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(−)-REGIOLONE, AN α-TETRALONE FROM *JUGLANS REGIA*: STRUCTURE, STEREOCHEMISTRY AND CONFORMATION

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Key Word Index—*Juglans regia*; Juglandaceae; α-tetralone; (−)-regiolone; structure; stereochemistry; conformation; juglone; betulinic acid; sitosterol.

Abstract—A new α-tetralone derivative designated (−)-regiolone has been isolated with juglone, betulinic acid and sitosterol from the stem-bark of *Juglans regia*. (−)-Regiolone has been shown to be 4,8-dihydroxy-1-tetralone on the basis of its spectral data and chemical transformation to juglone. Its conformation has been deduced from the ^{1}H NMR spectral data. The absolute stereochemistry of its only chiral centre has been shown to be *S* by the application of the dibenzoate chirality rule.

INTRODUCTION

Juglans regia L., the walnut tree, grows all through the Himalayas in India and Nepal at altitudes between 1000 and 3000 m [1]. Extracts of different parts of the plant have been used in folk medicine [1, 2]. A number of naphthalene derivatives were reported, e.g. juglone [3], 3,3′-bisjuglone [3, 4] and trijuglone [4, 5] from the root bark; α-hydrojuglone glucoside [6, 7] 1,4-naphthaquinone [7], naphthaquinol glucoside [7] from woody parts and leaves. Additionally, 4-hydroxy-1-naphthalenyl-α-D-glucopyranose [7] was isolated from the fruits of this and other *Juglans* species.

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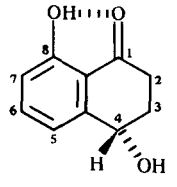
RESULTS AND DISCUSSION

Extensive silica gel chromatography of the chloroform extract of the defatted stem-bark of *Juglans regia*, collected from Kathmundu, Nepal, led to the isolation of a new tetralone (−)-regiolone (**1**) in addition to three known compounds *viz.*, sitosterol, juglone (**3**) and betulinic acid.

(−)-Regiolone, mp 72°, $C_{10}H_{10}O_3$ (M^+ 178), $[\alpha]_D$ −3.3° has been assigned structure (**1**) from spectral evidence and chemical transformation. The IR (KBr) bands at 1635 cm^{-1} (H-bonded CO), 3200–3350 (*br*, chelated OH) and 745 (1,2,3-trisubstituted benzene) together with its UV spectral characteristics [λ_{max}^{EtOH} nm (log ε) 216 (4.38), 259 (4.17), 333 (3.77); $\lambda_{max}^{EtOH/OH^-}$ 229 (4.25), 240 sh (4.22), 259 sh (3.94), 350 (3.69) and 372 (3.81)] are reminiscent of the presence of an *ortho*-hydroxyacetophenone type moiety in the molecule.



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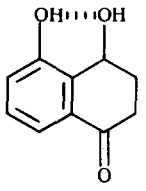

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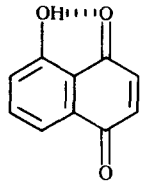

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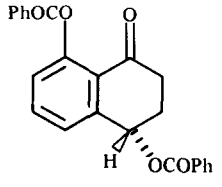

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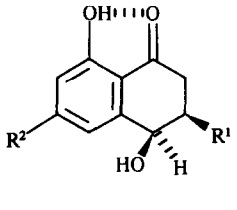

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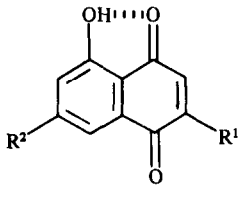

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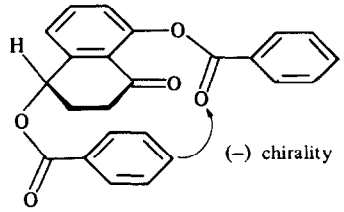


4a ≡ **4**

A 400 MHz ^{1}H NMR spectrum of regiolone (**1**) in $CDCl_3$ yielded complete structural and stereochemical information. In the aromatic region three vicinal protons appeared at δ6.91 (1H, *dd*, $J_{7,6}=7.7$ Hz, $J_{7,5}=1.3$ Hz, H-7), 7.01 (1H, *dt* $J_{5,6}=7.7$ Hz, $J_{5,7}=1.3$ Hz, $J_{5,4}=1.0$ Hz; H-5), 7.48 (1H, *t*, $J_{6,5}=J_{6,7}=7.7$ Hz, H-6), one chelated phenolic proton at δ12.35 (1H, *s*, 8-OH), a secondary carbinyl methine proton at δ4.92 (1H, 5 broad line *m*, $W_{1/2}=16.6$ Hz, H-4), two non-equivalent ketomethylene protons at δ2.91 and 2.63 (1H, *ddd* each, $J_{gem}=18.3$ Hz, $J_{trans}=8.7$ Hz, $J_{cis}=4.8$ Hz; H-2α, and H-2β respectively) and two non-equivalent methylene proton multiplets at δ2.29–2.37 (1H, *dddd* appearing as a 12 line *m*, H-3β) and 2.13–2.22 (1H, *dddd* appearing as a 13 line *m*, H-3α)–the assignments of the C-2 and C-3 protons are based on arguments stated later. The phenolic OH, aromatic protons and H-4 showed distinctly different chemical shift values from those of sclerone (2) [8]. Oxidation of regiolone (**1**) with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in benzene afforded juglone (**3**), thus supporting the formulation **1** for regiolone.

In order to determine the absolute configuration of the chiral centre C-4 of (−)-regiolone it was converted to its dibenzoate (**4**), mp 130°, $[\alpha]_D-122.6°$ and the aromatic chirality method [9, 10] was applied though there exists some limitations in the presence of a third chromophore (acetophenone moiety). (−)-Regiolone dibenzoate (**4**) in its CD curve exhibited a strong negative Cotton effect around 230 nm (Fig. 1) indicating a negative chirality (left-handed screw) in the dibenzoate as shown in (**4a**) as far as the application of the method is tenable. The CD curve also showed a weaker negative Cotton effect around 275 nm (Fig. 1) thus making a CD couplet. The absolute configuration at C-4 in (−)-regiolone is thus assigned (*S*) (C_4-OH α), as shown in formula **1**, in accordance with the negative chirality causing a negative Cotton effect. Application of the aromatic chirality method in natural tetralones shinanolone (**5**) [11] and isoshinanolone (**6**) [12] has been reported. This information is however, empirical, since in addition to the strong transition moments due to three interacting chromophores the preferred conformation of each benzoyloxy group which would be responsible for the direction of the transition moment is not definitely known; the conformation of the C_4-benzoyloxy group is an average of the pseudoaxial and pseudoequatorial orientations as would be evident from the careful analysis of the high resolution NMR data, discussed in the sequel. However, the chirality of (−)-regiolone could have been deduced unambiguously from the CD studies of the C_4-monobenzoate or the reduction product of the dibenzoate **4** which lacks the acetophenone chromophore. These products could not be prepared due to the paucity of regiolone.

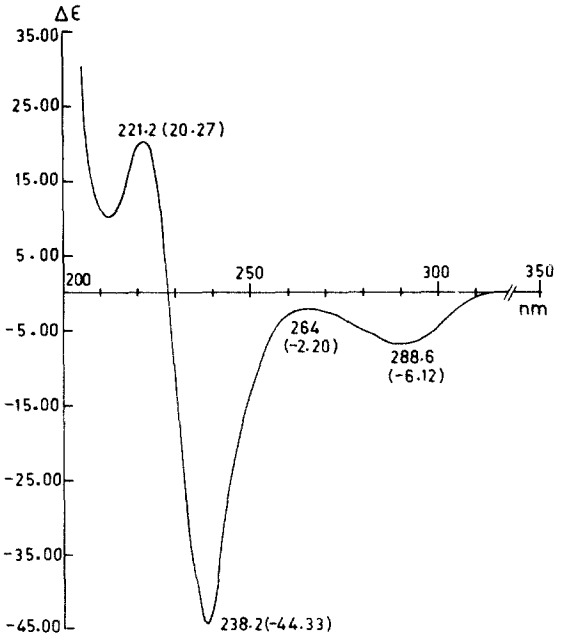
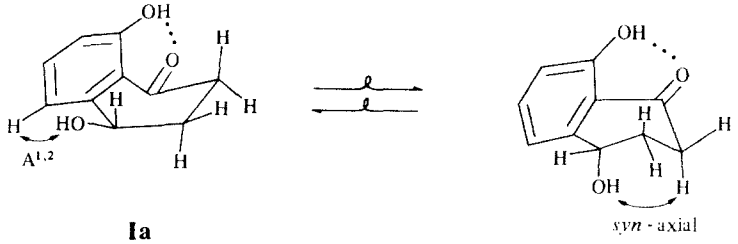


Fig. 1. CD spectrum of (−)-regiolone dibenzoate (**4**) (MeCN, *c* 0.125 mg/g).

The conformation of the cyclohexenone ring of regiolone was deduced from its high resolution ^{1}H NMR spectral data. A careful examination with Dreiding models revealed that the cyclohexenone ring could adopt either of the two nearly half-chair forms **1a** and **1b** having similar free energy, and hence about same population, because of the same type of H-bonded stabilisation and non-bonded interactions of nearly the same order, as indicated in **1a** and **1b**. The keto-methylene protons at C-2 appeared, as stated earlier, as a *ddd* with the same coupling constants but possessed different chemical shifts. The former observation revealed that conformational inversion at room temperature must be taking place in solution at a sufficiently fast rate and the observed conformation is 'time averaged' and represents the average of the participating conformational species **1a** and **1b** of equal energy in the inversion cycle thereby recording the average chemical shift of each methylene proton and the average J_{trans} (ee, aa) and J_{cis} (ea, ae) of each proton at C-2 (H-2α or H-2β) become equal. This is expected since the energy barrier between the two conformers **1a** and **1b** is too low not only to block the conformational inversion at room temperature but also to make the rate of inversion slow enough to enable the recording of signals of the particular protons of each



1a **1b**

conformer. The different chemical shifts of H-2α and H-2β were caused by different environments. Thus H-2α being equatorial in **1a** experienced deshielding effect of C=O and being axial in **1b** suffered deshielding caused by axial C_4-OH (α-); whereas H-2β experienced deshielding effect of C=O only in **1b**, (being equatorial), but no deshielding effect in (**1a**). Again, the two conformers were present in nearly equal proportions. Thus, H-2α was more deshielded compared to H-2β. Each nonequivalent methylene proton at C-3 experienced coupling with H-4β, H-2α and H-2β, in addition to the geminal coupling. Thus, H-3α experienced two diaxial vicinal couplings with H-2β and H-4β in **1a** whereas H-3β had only one diaxial coupling with H-2α in **1b** and all other couplings are of ae or ee in nature and hence of less J values. Consequently, the more shielded 13-line multiplet at δ2.13–2.23 with more splitting and more spread of the signals must be assigned to H-3α; hence the 12-line multiplet at δ2.29–2.37 is assigned to H-3β. Presumably, H-3α, being gauche to C_4-OH in both the conformers was more shielded than H-3β which is gauche to C_4-OH in conformer **1a** but anti to C_4-OH in conformer **1b**.

The structure of regiolone (**1a**) is fully consistent with its ^{13}C NMR spectrum in which all the carbon signals could be unambiguously assigned with the help of DEPT spectra (Experimental). The mass fragmentation pattern of regiolone, shown in Scheme 1, is in good agreement with its assigned structure **1**.

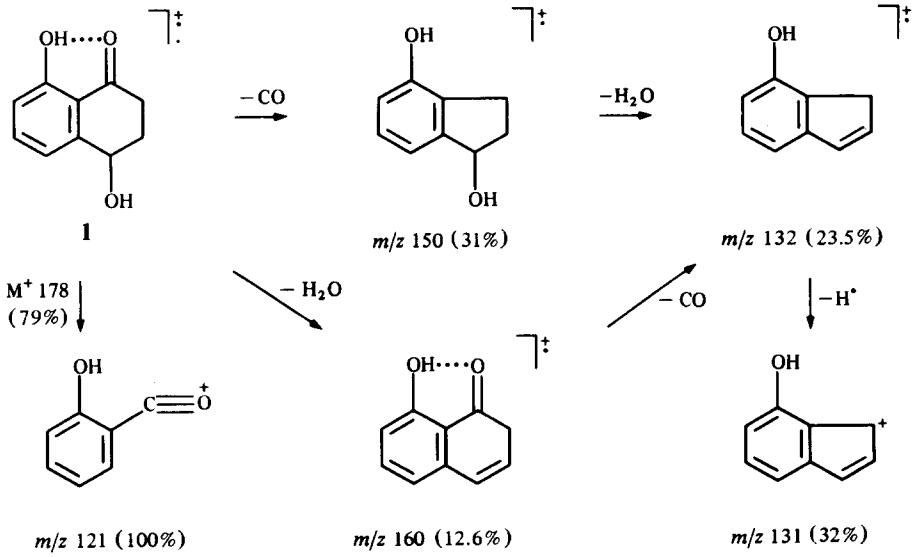
The co-occurrence of l-tetralones and the corresponding naphthaquinones, *viz.* shinanolone (**5**) and 7-methyljuglone (**7**) [11] as well as isoshinanolone (**6**) and plumbagin (**8**) [12] and in the present case regiolone (**1**) and juglone (**3**) is of biogenetic significance. Unlike **5**, **6** **7** and **8** which are derived by the polyketide pathway, juglone was shown [13] to be formed from shikimic acid all the seven carbon atoms of which are retained; the remaining three carbons originate from the three central carbons in glutamic acid or 2-ketoglutaric acid. Thus, regiolone is also considered to be derived from shikimic acid and is perhaps formed in the plant directly and not via **3**. However, this speculative biogenetic pathway needs confirmation through labelling experiments.

EXPERIMENTAL

General. Mps: uncorr. IR: KBr. UV: 95% EtOH. Chromatography: silica gel (BDH, 60–120 mesh); spots visualized on exposure to I_2 vapour; homogeneity established by TLC; light petrol bp 40–60°; ^1H NMR: TMS as int. standard; MS: 70 eV.

Extraction. Dried and powdered stem-bark (2 kg) of *Juglans regia* collected from Phulchowki (1800 m alt.), Kathmundu, Nepal, was exhaustively extracted in a Soxhlet successively with petrol (bp 60–80°) and $CHCl_3$ for 48 hr each. The marc left was extracted with cold MeOH. Each extract was concd under red. pres. The residues obtained from the petrol and MeOH extracts did not give any crystalline or pure compound even after careful column or thin layer chromatography. The dark brown gummy residue (22 g) obtained from the $CHCl_3$ extract was chromatographed over silica gel (430 g), elution being continued with solvents and solvent mixtures of increasing polarity. Fractions of similar composition (as indicated by TLC) were combined.

Isolation of juglone, sitosterol and betulinic acid. The residue obtained from the earlier petrol–EtOAc (19:1) eluate fractions of the main column on repeated chromatography yielded juglone, crystallizing from $CHCl_3$ in deep orange-red needles (10 mg), mp 156° (dec.); R_f 0.45 in C_6H_6–petrol (1:1) on TLC slide; the same spot was shown by the original $CHCl_3$ extract indicating that (**3**) was not an artefact derived from regiolone (**1**); IR ν_{max} cm^{-1} 3470 (–OH), 1660 (C=O), 1640 (chelated C=O); ^1H NMR: (δ400 MHz, $CDCl_3$) 6.95 (2H, *s*, H-2 and H-3), 7.27 (1H, *dd*, J = 7.5 Hz and 2.5 Hz, H-6), 7.63 (2H, *m*, H-7 and H-8) and 11.89 (1H, *s*, chelated OH). The residue obtained from the later petrol–ethyl acetate (19:1) eluate fractions upon rechromatography over silica gel afforded sitosterol [14] (50 mg), colourless needles (from petrol), mp 137° $[\alpha]_D$ –37° ($CHCl_3$); acetate (Ac_2O/pyridine, room temp. 20 hr), mp 134°, $[\alpha]_D$ –30.5 ($CHCl_3$). The residue obtained from the earlier petrol–EtOAc (9:1) eluate fractions of the main chromatogram on rechromatography furnished betulinic acid [15] (80 mg), colourless feather like needles (from MeOH–$CHCl_3$), mp 290°, $[\alpha]_D$ +11° ($CHCl_3$);



Scheme 1. Mass fragmentation of regiolone (**1**).

IR: ν_{max} cm^{-1} 3440 (OH of COOH), 2940, 2260 (O-H), 1685 (CO of COOH), 1450, 885; acetate (Ac_2O/pyridine, room temp., overnight), mp 292° (colourless flakes from light petrol–$CHCl_3$ mixture); methyl ester (CH_2N_2–ether), mp 208° (colourless needles From petrol–$CHCl_3$ mixture), $[\alpha]_D$ +7.5° ($CHCl_3$).

Isolation of (−)-regiolone (**1**). The residue obtained from the later petrol–EtOAc (9:1) eluate fractions of the main chromatogram on several rechromatographies furnished colourless crystals from the petrol–EtOAc (9:1) eluate fractions which were recrystallized from petrol as fine short needles (52 mg), mp 72° [R_f 0.27 in $CHCl_3$–MeOH (97:3), silica gel], $[\alpha]_D$ −3.3° (EtOH; *c* 0.077) deep violet colouration with $FeCl_3$ (neutral); ^{13}C NMR (75.47 MHz, $CDCl_3$): δ31.24 (C-3), 34.55 (C-2), 67.72 (C-4), 115.30 (C-8a), 117.35 (C-7), 117.77 (C-5), 136.93 (C-6), 145.93 (C-4a), 162.78 (C-8) and 204.21 (C-1). (Found: C, 67.07; H, 5.48. $C_{10}H_{10}O_3$ requires: C, 67.42; H, 5.62%).

Oxidation of regiolone (I) *to juglone* (III). A mixture of (−)-regiolone (20 mg) and 2,3-dichloro-5,6-dicyanobenzoquinone (50 mg) taken in dry C_6H_6 (3 ml) was refluxed to 8 hr. The mixture was then concd to 2 ml and purified by prep. TLC to obtain deep orange-red crystals of juglone (10 mg), mp 155°, identified by its direct comparison (IR, co-TLC, mmp) with the natural sample.

Regiolone 4,8-dibenzoate (**4**). Regiolone (4 mg) was dissolved in pyridine (0.1 ml) and to this solution was added freshly distilled benzoyl chloride (0.1 ml). The mixture was warmed for 3 min and kept at room temperature for two days. After the usual work-up, the residue crystallized from $CHCl_3$–petrol, mp 130 (2.5 mg), $[\alpha]_D$ −122,6° ($CHCl_3$; *c* 0.043). UV λ_{max} nm (log ε) 211 (4.59), 233 (4.69), 275 (3.59), 283 (3.61); IR ν_{max} cm^{-1} 3060, 2950 (CH_2), 1740, 1705 (benzoate C=O), 1682 (CO), 1600, 1570, 1490 (aromatic absorption), 860, 800, 750 (1,2,3-trisubstituted benzene); ^1H NMR ($CDCl_3$, 300 MHz): δ8.23 (1H, *dd*, J = 7.7 Hz and 1.4 Hz, H-5), 8.05 (1H, *dd*, J = 7.9 Hz, 1.4 Hz, H-7), 7.27 (1H, *t*, J = 7.7 Hz, H-6), 7.54 (10 H, *m*, 10 aromatic protons of the two benzoate moieties). 6.41 (1H, *m*, H-4), 2.96 (1H, *m*, H-2β), 2.70 (1H, *m*, H-2α), 2.48 (2H, *m*, H_2-3).

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