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CYCLOHEXANOLS OF *HALLERIA LUCIDA*

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Key Word Index—*Halleria lucida*; Scrophulariaceae; *trans*-1-(2′-hydroxyethyl)cyclohexan-1,4-diol; 4-hydroxy-4-(2′-hydroxyethyl)cyclohexanone; ^{13}C NMR.

Abstract—From the stems of *Halleria lucida* a new 1,4-cyclohexandiol, a *trans*-1-(2′-hydroxyethyl)cyclohexan-1,4-diol named isorengyol, together with the known *cis* isomer rengyol, was isolated. The leaves of the same plant contain acteoside, rutin, luteolin-5-*O*-glucoside and a 4-hydroxy-4-(2′-hydroxyethyl)cyclohexanone, of which the hemiketalic tautomer has recently been described.

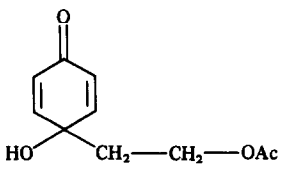
INTRODUCTION

In a previous paper, some of us [1] reported the isolation from the leaves of *Halleria lucida*, an African perennial plant utilized in traditional medicine, of a cyclohexadienone and of a cyclohexenone, named hallerone (**1**) and halleridone (**2**), respectively. We now report the isolation of a new cyclohexandiol, **3**, from the stems of the same plant and of the corresponding cyclohexanone, **4**, from the leaves, together with a known phenylpropanoid glucoside, acteoside, and two flavonoids, rutin and luteolin-5-*O*-glucoside. The last compound has already been reported from the same plant [2].

RESULTS AND DISCUSSION

By counter-current distribution (CCD) of the butanolic extract obtained after exhaustive extraction of *H. lucida* stems with methanol, compound **3** was isolated as the main component (0.005%). It is a viscous oil, $C_8H_{16}O_3$, *m/z* 142 $[M-18]^+$ (24%), which exhibited a broad IR band at 3500 cm^{-1}, whereas it was transparent in the UV range of the spectrum and lacked significant rotatory power in various solvents.

The ^{1}H NMR spectrum of **3** in CD_3OD showed signals characteristic of an A_2X_2 system at δ1.96 and 3.96 (triplets, $J = 7$ Hz) assignable to the sequence $-\overset{|}{C}-CH_2-CH_2O-$ confirmed by the signals at 41.3 and 58.8 ppm, both triplets in the ^{1}H-coupled ^{13}C NMR spectrum. After acetylation **3** gave the corresponding diacetyl derivative **5**, $C_8H_{14}O_3(Ac)_2$, wherein the signal at δ3.96 in **3** was shifted downfield (δ4.28). This accounted for the presence of a β-hydroxyethyl group, in agreement with the loss of 45 mu in the mass spectrum of **3** (*m/z* 115, 41%). Furthermore, by comparison of the ^{1}H NMR spectra of **3** and **5** the presence of a secondary hydroxyl group in **3** was established (1H, multiplet at δ3.93 in **3** and 4.98 in **5**) and



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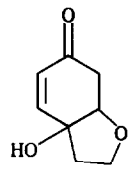

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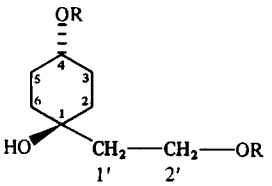

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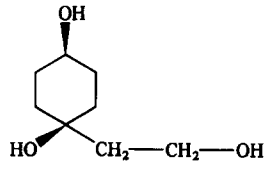
5 R = Ac



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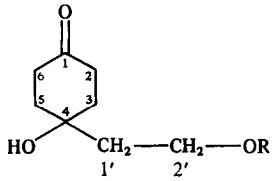

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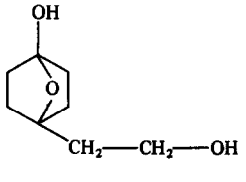
7 R = Ac



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confirmed by the signal of the corresponding carbon atom (doublet in the ^{1}H-coupled ^{13}C NMR spectra of **3**, at 68.0 in CD_3OD and at 67.4 ppm in C_5D_5N) (See Table 1).

The absence of further signals at low field in the ^{1}H NMR spectra of **3** and **5**, besides the presence of a quaternary carbon atom at 71.3 ppm and of four methylenic groups (two to two equivalent) at 30.3 and 33.9 ppm in the ^{13}C NMR spectrum of **3** in CD_3OD, accounted for the presence of a tertiary hydroxyl group and therefore of a 1,4-disubstituted cyclohexane ring, in agreement with the lack of optical rotation.

On the basis of all the aforementioned data, the structure of the 1-(2'-hydroxyethyl)cyclohexan-1,4-diol was assigned as **3**. The equatorial orientation of H-4 was established on account of the half-height width (8 Hz) of the corresponding signal in the ^{1}H NMR spectrum of **5** (in **3**, the signals of H-4 and H_2-2' are partially overlapped). The equatorial orientation for the β-hydroxyethyl group, assumed on the basis of energetic considerations, suggested for the compound the structure **3** with a *trans* relationship between the two hydroxyl groups in the 1 and 4 positions.

Compound **3** is thus an isomer of rengyol (**6**), recently isolated from *Forsythia suspensa* Vahl (Oleaceae), and for it the name of isorengyol can be assumed [3]. The *cis* relationship between the two hydroxyl groups in the 1 and 4 positions for rengyol was assigned on the basis of the broad half-height width (20 Hz) of H-4. There is a remarkable downfield shift of H-4 in **3** and **5** with respect to the corresponding axially oriented hydrogen in **6** and in its diacetyl derivative ($\Delta\delta = 0.4$ and 0.32, respectively).

In the ^{1}H and ^{13}C NMR spectra of the sample of isorengyol isolated from *H. lucida* the presence of some minor signals, identical to those reported for rengyol, suggested a further purification which resulted in the isolation of a small amount of this compound, for which all the spectroscopic data were found to be identical to those reported for **6**.

With regard to the leaves of *H. lucida*, the methanolic extract was partitioned between water and, in order, cyclohexane, ethyl acetate (in this solvent, luteolin-5-*O*-glucoside [2] was recovered as an insoluble) and *n*-butanol. By CCD of the butanolic extract two compounds were isolated and identified as rutin [4] and acteoside [5] whereas by CCD of the ethyl acetate extract a viscous oil, **4**, $C_8H_{14}O_3$, *m/z* 158 $[M]^+$ (21%), was obtained besides hallerone (**1**) and halleridone (**2**). Compound **4** is optically inactive and the IR spectrum in $CHCl_3$ showed absorption bands at 1710 cm^{-1} (C=O) and the region between 3400 and 3500 cm^{-1} (OH), whereas the UV spectrum in MeOH showed a weak band at 287 nm.

The ^{1}H NMR spectrum of **4** in $CDCl_3$ (see Experimental) showed the signals of an ABCD system corresponding to eight hydrogens assignable to four methylenic groups, two to two equivalent, and the signals of an A_2X_2 system at δ 1.68 and 3.90 (triplets, J = 8.0 Hz) The last signal was shifted downfield (δ4.20) in the corresponding monoacetyl derivative, $C_8H_{13}O_3Ac$ (**7**), and this accounted for the presence of the sequence $-\overset{|}{C}-CH_2-CH_2OH$ in **4** as in **3**. The second oxygen of **4** was assigned to a non-conjugated carbonyl group on the basis of (i) the IR stretching band at 1710 cm^{-1}, (ii) the UV absorption band at 287 nm, and (iii) the signal at 211.2 ppm in the ^{13}C NMR spectrum in $CDCl_3$ and 211.3 ppm in C_5D_5N. Likewise, as for **1** the third hydrogen pertains to a tertiary hydroxyl group and therefore the 4-hydroxy-4-(2'-hydroxyethyl)-cyclohexanone structure could be assigned to **4**. Thus the compound is a tautomer of rengyoxide, previously isolated from *F. suspensa* [3] and for which the hemiketalic structure **8** was assigned on the basis of the spectral data. However, by comparison with an authentic specimen, the spectroscopic data found by us, identical for the two samples, correspond to the hydroxyketonic form, **4**, probably the more stable one, present also in the monoacetyl derivative **7** (see Table 1).

The co-occurrence of strictly related compounds, such as hallerone (**1**), halleridone (**2**), isorengyol (**3**), rengyol (**6**) and rengyoxide (**4**), in the same plant suggests a biogenetic relationship. Furthermore, the occurrence of these cyclohexanols, structurally similar to prephenic acid, together with phenylpropanoid glucosides, already observed in *F. suspensa* [3] and *Calceolaria* species [6] suggests their involvement in the biogenesis of the aromatic compounds of these plants.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were measured at 400 and 100 MHz, respectively. Separations were performed by CCD with a Craig Post apparatus (200 stages, 10:10 ml, upper and lower phases) and monitored by TLC on silica gel F_{254}. The spots were detected by spraying with anisaldehyde–H_2SO_4 reagent.

Plant material. Leaves and stems of *H. lucida* L. were collected in the Botanical Garden of Rome in December 1984.

Extraction and separation. Stems (5 kg) were ground and extracted × 3 with MeOH. After evaporation of solvent the

Table 1. ^{13}C NMR chemical shift assignments of compounds **3**–**7** (TMS as internal standard)

C	**3** (CD_3OD)	**3** (C_5D_5N)	**5** ($CDCl_3$)	**6** (CD_3OD)	**6** (C_5D_5N)	**4** (C_5D_5N)	**4** ($CDCl_3$)	**7** ($CDCl_3$)
1	71.3	71.2	n.o.	70.7	69.9	211.3	211.2	211.5
2, 6	33.9	34.4	32.8	35.5	36.0	37.4*	35.4*	36.7*
3, 5	30.3	31.0	26.0	30.6	31.6	37.7*	35.6*	37.0*
4	68.0	67.4	69.0	70.0	69.7	69.7	69.0	69.2
1'	41.3	43.0	40.3	43.9	45.1	43.9	40.3	40.3
2'	58.8	58.7	60.7	58.8	58.7	58.7	57.9	60.6
C=O			170.6, 171.0					170.7
Me			21.0, 21.3					20.9

*These signals may be reversed.
n.o., Not observed.

residue (15 g) was partitioned between H_2O–EtOH–EtOAc–cyclohexane (10:4:2:11) and the upper phase containing non-polar compounds discarded. The aq. phase after evaporation of EtOH was repeatedly extracted with *n*-BuOH. The residue of the combined organic phases was submitted to CCD between H_2O–EtOAc–*n*-BuOH (10:9:1) affording compound **3** (0.25 g, $K_r = 0.5$).

Fresh leaves (0.45 kg) were shredded and extracted × 3 with MeOH. After evaporation of solvent the residue (58 g) was partitioned between H_2O (500 ml) and, in order, cyclohexane (2 × 300 ml), EtOAc (2 × 300 ml) and *n*-BuOH (2 × 300 ml). The cyclohexane extract, containing mostly chlorophylls (21 g), was not examined whereas the EtOAc residue (6.5 g) was fractionated by CCD between H_2O–EtOH–EtOAc–cyclohexane (5:2:5:2). Besides the known hallerone (**1**) and halleridone (**2**), identified by direct comparison with authentic specimens, a viscous oily compound, **4** (0.36 g, $K_r = 0.16$) was obtained. During the partition between H_2O and EtOAc a fine powder separated. This was recovered by filtration, washed with EtOAc, crystallized from H_2O and identified as luteolin-5-*O*-glucoside. The *n*-BuOH residue (7.6 g) was submitted to CCD between H_2O–EtOAc–*n*-BuOH (25:23:2) giving two compounds which were identified as acteoside (1.4 g, $K_r = 0.67$) and rutin (0.8 g, $K_r = 0.45$).

Trans-1-(2'-hydroxyethyl)cyclohexan-1,4-diol, isorengyol (**3**). Oil. (Found: C, 59.52; H, 10.38. $C_8H_{16}O_3$ requires: C, 59.98; H, 10.07%.) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (broad). ^1H NMR (CD_3OD): δ 1.6–2.0 (8H, H_2-2, H_2-3, H_2-5 and H_2-6), 1.96 (2H, *t*, $J_{1',2'} = 7$ Hz, H_2-1'), 3.93 (1 H, *m*, H-4), 3.96 (2 H, *t*, H_2-2'). ^{13}C NMR: see Table 1. MS *m/z* (rel. int.): 142 (24), 115 (41), 102 (97), 97 (100), 84 (64), 83 (48), 54 (75).

Rengyol (**6**). By prep. TLC on silica gel 60 plates (with H_2O saturated *n*-BuOH) rengyol was obtained suitably pure from the aforementioned sample of isorengyol. It was identified by its spectroscopic data (see Table 1) and by comparison with data reported in the lit. [3].

Diacetylisorengyol (**5**). Compound **3** was acetylated with Ac_2O–pyridine at room temp. overnight. After evaporation of reagents *in vacuo* and CCD of the residue between H_2O–Me_2CO–EtOAc–cyclohexane (5:6:1:7), **5** ($K_r = 0.6$) was obtained. Oil. (Found: C, 58.78; H, 8.61. $C_{12}H_{20}O_5$ requires: C, 59.00; H, 8.25%.) ^1H NMR ($CDCl_3$): δ 1.4–1.9 (8H, H_2-2, H_2-3, H_2-5 and H_2-6), 1.86 (2H, *t*, $J_{1',2'} = 7$ Hz, H_2-1'), 2.01 and 2.03 (2 × 3H, 2*s*, 2Ac), 4.28 (2H, *t*, H_2-2'), 4.98 (1H, *m*, $W_{1/2} = 8$ Hz, H-4). MS *m/z* (rel. int.): 184 $[M - HOAc]^+$ (8), 157 (34), 125 (19), 124 (94), 97 (92), 83 (35), 70 (72), 43 (100). ^{13}C NMR: see Table 1.

4-Hydroxy-4-(2'-hydroxyethyl)cyclohexanone (**4**). Oil. (Found: C, 60.34; H, 8.62. $C_8H_{14}O_3$ requires: C, 60.74; H, 8.92%.) UV λ_{max}^{MeOH} nm (log ε): 287 (2.4). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500–3400, 1710. ^1H NMR ($CDCl_3$): δ 1.61 (2H, *ddd*, $J_{gem} = 16.0$, $J_{e,a} = 5.0$, $J_{a,a} = 14.0$ Hz, H-3a and H-5a), 1.68 (2H, *t*, $J_{1',2'} = 8.0$ Hz, H_2-1'), 2.00 (2 H, *ddd*, $J_{gem} = 16.0$, $J_{e,e} = 6.5$, $J_{e,a} = 7.0$ Hz, H-3e and H-5e), 2.11 (2H, *ddd*, $J_{gem} = 14.5$ Hz, H-2e and H-6e), 2.64 (2H, *ddd*, H-2a and H-6a), 3.33 (2H, exch. D_2O, 2OH), 3.90 (2H, *t*, H_2-2'). ^{13}C NMR: see Table 1. MS *m/z* (rel. int.): 158 $[M]^+$ (21), 140 (37), 113 (42), 101 (80), 86 (70), 83 (67), 55 (100).

4-Hydroxy-4-(2'-acetoxyethyl)cyclohexanone (**7**). Compound **4** was acetylated with Ac_2O–pyridine at room temp. overnight. After evaporation of reagents *in vacuo* and purification of the residue by CCD between H_2O–EtOH–EtOAc–cyclohexane (5:2:4:3), **7** ($K_r = 0.3$) was obtained. Oil. (Found: C, 59.73; H, 8.10. $C_{10}H_{16}O_4$ requires: C, 59.98; H, 8.05%.) ^1H NMR ($CDCl_3$): δ 1.66 (2H, *ddd*, $J_{gem} = 16.0$, $J_{e,a} = 5.0$, $J_{a,a} = 14.0$ Hz, H-3a and H-5a), 1.78 (2H, *t*, $J_{1',2'} = 8.0$ Hz, H_2-1'), 1.92 (2H, *ddd*, $J_{gem} = 16.0$, $J_{e,a} = 7.0$, $J_{e,e} = 6.5$ Hz, H-3e and H-5e), 1.92 (3H, *s*, Ac), 2.16 (2H, *ddd*, $J_{gem} = 14.5$ Hz, H-2e and H-6e), 2.61 (2H, *ddd*, H-2a and H-6a), 4.20 (2H, *t*, H_2-2'). ^{13}C NMR data: see Table 1. MS *m/z* (rel. int.): 200 $[M]^+$ (2), 140 (34), 122 (18), 85 (23), 43 (100). Rutin [4] and acteoside [5] were identified by direct comparison with authentic specimens. Luteolin-5-*O*-glucoside was identified by comparison of the chemical and physical data with those in the lit. [2].

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