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Preparation and Relative Configurations of Some 2,2'-Spirobiindanes

E. DYNESEN

Department of Chemistry, University of Aarhus, DK-8000 Aarhus C, Denmark

Stepwise reduction of 2,2'-spirobi[2*H*-indene]-1,1'-(3*H*,3'*H*)-dione (*1*) according to Scheme 1 gives two diastereomeric monohydroxy ketones (2*a*,*b*) and three diastereomeric dihydroxy compounds (3*a*,*b*,*c*); formulae, see Fig. 1 and Table 1.

Scheme 1.

The monohydroxy ketone (2*b*) and the dihydroxy compound (3*b*) were previously prepared¹ by reduction of *1* with lithium tri-*t*-butoxyaluminium hydride [LiAl(O-*t*-Bu)₃H] and lithium aluminium hydride, respectively, in tetrahydrofuran (THF).

In continuation of these experiments, *1* was reduced with excess of LiAl(O-*t*-Bu)₃H in THF. The product was a mixture of the epimeric dihydroxy compounds (3*a*) and (3*b*) of which 3*a* was isolated in 60–65 % yield.

Reduction of 2*b* with LiAl(O-*t*-Bu)₃H in THF afforded a mixture of preferentially 3*b* and 3*c*.

Fig. 1.

Table 1. ¹H NMR data ^a for some 2,2'-spirobiindane derivatives in DMSO-*d*₆ solution.

Signal	δ^b	δ_{AB}^c	J_{AB}/Hz^c
4,² R¹=R²=R³=R⁴=H			
CH ₂	2.89		
5,² R¹,R²=O; R³=R⁴=H			
H(1')=H(3')	3.08	0.39	15.5
H(3)	3.18		
6, R¹=R²=H; R³,R⁴=H,OH			
H(1) ^d	2.85	<i>f</i>	<i>f</i>
H(1') (H)	4.78	0.60	6.0
(OH)	5.38		
H(3') ^d	2.86	<i>f</i>	<i>f</i>
H(3)	2.93	0.83	16.0
2a, R¹,R²=O; R³,R⁴=OH,H			
(H1') (H)	5.01	0.42	6.5
(OH)	5.43		
H(3')	3.12	0.44	16.0
H(3)	3.23	0.08	17.9
2b, R¹,R²=O; R³,R⁴=H,OH			
H(1') (H)	5.33	0.45	6.3
(OH)	5.78		
H(3')	3.03	0.32	15.4
H(3)	3.21	0.92	17.3
3a, R¹,R²=H,OH; R³,R⁴=OH,H			
H(1)=H(1') (H)	4.99	0.43	4.2
(OH)	5.42		
H(3)=H(3')	2.73	0.62	15.5
3b, R¹,R²=H,OH; R³,R⁴=H,OH			
H(1) ^e (H)	4.59	0.55	6.5
(OH)	5.14		
H(1') ^e (H)	5.27	0.48	6.5
(OH)	4.79		
H(3)	3.00	0.90	16.0
H(3')	2.77	<i>f</i>	<i>f</i>
3c, R¹,R²=OH,H; R³,R⁴=H,OH			
H(1)=H(1') (H)	5.01	0.46	6.2
(OH)	5.47		
H(3)=H(3')	2.79	0.81	15.6

^a The spectra were recorded at 60 MHz except for compound 2*a* which was recorded at 100 MHz. TMS was used as internal reference. ^b For the methylene groups the quoted values are the arithmetic means of the chemical shifts of the A and B protons. ^c δ_{AB} and J_{AB} are the differences in chemical shift between protons A and B, and the corresponding coupling constants, respectively. ^{d,e} Assignments may be reversed. ^f Not calculated because of lack of fine structure.

The yield of **3c** was 65–70 %. The NMR spectra of **3b** and **3c** demonstrated that these two dihydroxy compounds were identical with those previously obtained by reduction of the *o*-camphanic ester of **2b** with LiAlH_4 in THF.¹

The dihydroxy compounds (**3a,b,c**) were characterized as their diacetates.

Partial reoxidation of the dihydroxy compounds by means of manganese dioxide demonstrated that **3a** and **3c** gave only a single monohydroxy ketone **2a** and **2b**, respectively, while **3b** afforded a mixture of **2a** and **2b**. **3a** could be differentiated from **3c** since **3a** formed an *O,O*-isopropylidene derivative on treatment with 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulfonic acid.

The relative configurations of the monohydroxy ketones (**2a,b**) and the dihydroxy compounds (**3a,b,c**) could be established by studying the ^1H NMR splitting pattern of the AB systems exhibited by the protons at the 1,1' and 3,3' positions, respectively, in compounds **2a**–**6**, (see Fig. 1 and Table 1).

The relative assignments of the secondary alcohol groups and the methylene groups, as well as the specification of the H and OH signals in the monohydroxy and dihydroxy compounds, were performed by deuteration of the hydroxyl groups.

The assignments of the monohydroxy ketones (**2a,b**) and the dihydroxy compounds (**3a,b,c**) were based mainly on the variation in the δ_{AB} parameters of the H(3) and H(3') methylene groups as a result of the different configurations of the secondary alcohol groups H(1') and H(1), respectively.

The chemical oxidations of **3a,b,c**, the formation of an *O,O*-isopropylidene derivative of **3a** and the NMR assignments according to Table 1 were consistent with the correlations depicted in Scheme 1. The isolation of **2b** and **3a** after reduction of **1** with deficiency and excess of $\text{LiAl}(\text{O}-t\text{-Bu})_3\text{H}$, respectively, was, however, incompatible with Scheme 1. The observation that **2a** could easily be rearranged to **2b** under alkaline conditions as well as the detection of small amounts of **2a** in the monoreduction of **1** with $\text{LiAl}(\text{O}-t\text{-Bu})_3\text{H}$ suggested that **2a** was the primary reduction product which epimerized to **2b** under the alkaline conditions used in the work-up procedure.

Experimental. NMR spectra were recorded on Varian A-60 and XL-100 spectrometers. IR spectra were recorded on a Perkin-Elmer model 521 spectrophotometer. Elemental analyses were performed by Novo Industry A/S, Copenhagen.

cis,cis-1,1',3,3'-Tetrahydro-2,2'-spirobi[2H-indene]-1,1'-diol (3a). A solution of **1** (3.72 g, 15 mmol) in THF (150 ml, distilled over LiAlH_4) was added to a stirred mixture of $\text{LiAl}(\text{O}-t\text{-Bu})_3\text{H}$ (15.27 g, $\geq 90\%$, Fluka) and THF (50 ml) at room temperature. The mixture was refluxed with stirring for 3 h. Ethyl acetate (15 ml) was added to the warm reaction mix-

ture. After the exothermic reaction had subsided, the mixture was cooled and H_2O (20 ml) added. Next day, the inorganic precipitate was filtered with suction and washed with warm CHCl_3 . The combined filtrates were evaporated and the crystalline residue was recrystallized from benzene (220 ml) to give **3a** (2.47 g, 65 %), m.p. 235.5–237.5 °C. Anal.: $\text{C}_{17}\text{H}_{18}\text{O}_2$: C, H. Evaporation of the filtrate from **3a** afforded two fractions, the first of which contained mainly **3b** (0.42 g).

trans,trans-Isomer (3c). The hydroxy ketone **2b** (1.0 g, 4 mmol) was added to a mixture of $\text{LiAl}(\text{O}-t\text{-Bu})_3\text{H}$ (4.07 g) and THF (54 ml). The mixture was stirred and refluxed for 3 h and then decomposed by addition of ethyl acetate (4 ml) and H_2O (5 ml). After work-up as described for **3a**, the product was recrystallized from benzene to give **3c** (0.71 g, 70 %), m.p. 154.5–155.5 °C. Anal.: $\text{C}_{17}\text{H}_{18}\text{O}_2$: C, H. Evaporation of the filtrate from **3c** afforded a crystalline residue which was shown by means of NMR and TLC to contain mainly **3b**.

Diacetyl derivatives of 3a,b,c. A mixture of the dihydroxy compound (1 mmol), acetic anhydride (10 mmol) and pyridine (2 ml) was left at room temperature for 2 days. The reaction mixture was concentrated *in vacuo* and the residue dissolved in benzene (5–10 ml). The benzene solution was washed successively with HCl (3 N), Na_2CO_3 (5 %) and saturated NaCl solution. After drying and evaporation of the solution to dryness, the residue was recrystallized from benzene-light petroleum to give the pure diacetate in 70–90 % yield. Diacetate of **3a**. M.p. 202–203.5 °C. Anal.: $\text{C}_{21}\text{H}_{20}\text{O}_4$: C, H. ^1H NMR (60 MHz, CDCl_3): δ 7.66–7.17 (8 H, m), 6.28 (2 H, s), 2.84 (4 H, dd, J 15 Hz), 1.94 (6 H, s). Diacetate of **3b**. M.p. 143–144 °C. Anal.: $\text{C}_{21}\text{H}_{20}\text{O}_4$: C, H. ^1H NMR (60 MHz, CDCl_3): δ 7.51–7.18 (8 H, m), 6.49 (1 H, s), 5.92 (1 H, s), 3.19 (2 H, dd, J 16 Hz), 2.97 (2 H, s), 2.06 (3 H, s), 1.94 (3 H, s). Diacetate of **3c**. M.p. 156–157.5 °C. Anal.: $\text{C}_{21}\text{H}_{20}\text{O}_4$: C, H. ^1H NMR (60 MHz, CDCl_3): δ 7.43–7.17 (8 H, m), 6.23 (2 H, s), 3.12 (4 H, dd, J 16 Hz), 1.85 (6 H, s).

O,O-Isopropylidene derivative of 3a. A mixture of **3a** (0.2 g), *p*-toluenesulfonic acid (0.02 g, dried at 60–70 °C), and 2,2-dimethoxypropane (4 ml) was left at room temperature for 45 h. CHCl_3 (10 ml) was added to the reaction mixture and the organic phase was washed successively with H_2O , KHCO_3 (5 %) and saturated NaCl solution. After drying (CaSO_4), the solution was evaporated and the residue (0.24 g) recrystallized from light petroleum to give the pure compound (0.13 g), m.p. 109–110.6 °C. Anal.: $\text{C}_{20}\text{H}_{20}\text{O}_2$: C, H. ^1H NMR (60 MHz, CDCl_3): δ 7.60–7.16 (8 H, m), 4.94 (2 H, s), 2.95 (4 H, dd, J 15.8 Hz), 1.48 (6 H, s). IR (CCl_4): 1228 cm^{-1} (C–O–C).

cis-1'-Hydroxy-1',3'-dihydro-2,2'-spirobi[2H-inden]-1(3H)-one (2a). A mixture of **3a** (1.01 g, 4 mmol) and CHCl_3 (80 ml), dried

over CaCl_2 and then distilled, was refluxed with stirring. When the mixture was homogeneous, MnO_2 (1.6 g) was added in one lot. Stirring under reflux was continued for 1 h. The warm mixture was filtered with suction through celite. Evaporation of CHCl_3 from the filtrate afforded a crystalline residue. The combined residues (3.5 g) from the oxidation of a total of 3.47 g of **3a** were recrystallized from benzene to give unchanged **3a** (2.19 g) and a crystalline residue (1.20 g), which was shown by TLC to be a mixture of the spirodiketone (**1**) and **2a**. This fraction was chromatographed on silica gel (24×2 cm) using benzene- CHCl_3 as eluent. Evaporation of the first fractions of eluate (85 ml) gave the spirodiketone **1** (0.14 g). A middle fraction (100 ml) contained a mixture of **1** and **2a**. Evaporation of the following fractions (140 ml) afforded mainly **2a** (0.75 g). Recrystallization of this fraction from ethyl acetate-light petroleum (1:3) gave pure **2a** (0.55 g); m.p. 121–122.5 °C. (Found: C 81.48; H 5.59. Calc. for $\text{C}_{17}\text{H}_{14}\text{O}_2$: C 81.58; H 5.64). IR (CCl_4): 1724 cm^{-1} ($\text{C}=\text{O}$).

Rearrangement of 2a. A mixture of **2a** (0.032 g, 0.125 mmol), ethanol (1.25 ml, 96 %), H_2O (0.75 ml) and aqueous KOH (0.5 ml, 0.5 N) was refluxed for 1 min. The reaction mixture which contained a precipitate was diluted with H_2O (5 ml) and then cooled. The yield of **2b** was 0.0265 g (84 %); m.p. 196.5–199.5 °C.

The product was identical with the monoreduction product (**2b**) of **1** (TLC and mixed m.p.).

1,1',3,3'-Tetrahydro-2,2'-spirobi[2H-indene]-1-ol (6). A mixture of NaBH_4 (0.12 g, 3 mmol), ethanol (7 ml, 96 %) and the spiroketone (**5**)⁴ (0.47 g, 2 mmol) was stirred at room temperature for 2 h. After cooling in ice, cold HCl (3 ml, 2 N) was gradually added followed by CHCl_3 . The organic phase was washed with water and saturated NaCl solution. After drying (CaSO_4), the CHCl_3 solution was evaporated, and the residue recrystallized from light petroleum to give **6** (0.40 g, 84 %), m.p. 70.2–73.4 °C. (Found: C 86.05; H 6.78. Calc. for $\text{C}_{17}\text{H}_{16}\text{O}$: C 86.40; H 6.83). IR (CCl_4): 3500 cm^{-1} (hydrogen bonded OH), 3660 cm^{-1} (free OH).

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The Molar Absorptivity of Horseradish Peroxidase

P.-I. OHLSSON and K.-G. PAUL

Department of Chemistry, University of Umeå, S-901 87 Umeå, Sweden

The absorption coefficient for the Soret band of HRP* has been given by various authors as $\epsilon_{\text{mM}} = 91 - 107$, and this uncertainty has hampered kinetic and stoichiometric analyses.^{1,2} HRP has also been stated not to obey Beer's law.³ The deviations were attributed to aggregations but no experimental support was given. We have redetermined the light absorption coefficients of HRP and present them together with some comments on storage and handling of HRP.

Materials and methods. HRP A2 (pI 3.9) and C2 (pI 8.8) were prepared as described⁴ and found to be homogeneous in disc electrophoresis and isofocussing. Samples of HRP were also purchased from Boehringer, Mannheim (grade I), Worthington Biochemical Corp., New Jersey (code HPOFF), Sigma Chemical Company, St. Louis (type IV), and Nutritional Biochemicals, Cleveland (catalogue No. 100963). The commercial preparations were dissolved in phosphate buffer pH 7.0, dialyzed overnight against distilled water, and centrifuged for 30 min at 48 000 g.

HRP concentrations were based on pyridine hemochromogen determinations. ϵ for the α -band of this heme derivative has been given as 34.7⁵ and 33.9⁶ $\text{mM}^{-1} \text{cm}^{-1}$, $\Delta\epsilon$ (α -band—minimum at ~ 540 nm) as 24.6⁵ and 24.5⁶ $\text{mM}^{-1} \text{cm}^{-1}$, and the ratio $A_{\alpha\text{-band}}/A_{\text{min}}$ as 3.46–3.50⁵ and 3.60⁶. The values 33.9 and 24.5 give identical results when used to assay unknown solutions whereas 34.7 and 24.6 do not. For this reason, and because of the higher ratio and the complete absence of a plateau at 610–640 nm in the hemochromogen spectrum in the recent study⁶ we have used the value $\Delta\epsilon = 24.5 \text{ mM}^{-1} \text{cm}^{-1}$ in the present investigation.

Cytochrome c (Sigma, type VI) was reduced with dithionite in 50 mM sodium phosphate, pH 7.0, and filtered through Sephadex G-25 medium. $\Delta\epsilon_{550 \text{ red-ox}} = 19.6^7 \text{ mM}^{-1} \text{cm}^{-1}$.

Citric acid "nach Sørensen" (Merck, Darmstadt) was used. Pyridine (Mallinckrodt) was distilled over KOH. Water was distilled twice in all-glass vessels. Buffers, of ionic strength 0.1 unless otherwise stated, were filtered through Sartorius 0.2 μ membrane filters immediately before use. Ammonium sulfate *p.a.* (Merck, Darmstadt) was used for storage of HRP solutions.

* HRP, horseradish peroxidase (E. C. 1. 11. 1. 7, donor: H_2O_2 oxidoreductase). RZ, $A_{402.5}/A_{280}$.