Arginine modification. Citrate synthase at 20 μN was reacted with 60 mM butanedione in 1 ml 0.1 M borate buffer pH 7.5.[14] The modification was stopped by gel filtration of the mixture on a column of Sephadex G-15 (Pharmacia), using 0.125 M borate pH 7.5 as eluent. An aliquot was treated similarly but without butanedione. The enzyme was assayed for residual citrate synthase activity [17] and methylcitrate synthase activity by incubation for 20 h as above. To prevent restoration of essential arginine groups, the latter reaction was performed in 0.1 M borate pH 7.5 instead of phosphate buffer.[18]

Acknowledgement. This work was supported by the Swedish Natural Science Research Council.

1. Ando, T., Rasmussen, K., Wright, J. M. and Nyhan, W. L. *J. Biol. Chem. 247* (1972) 2200.
2. Brandänge, S., Josephson, S., Mörch, L. and Vallén, S. *Acta Chem. Scand. B 31* (1977) 307.
3. Stern, J. R. In Boyer, P. D., Lardy, H. and Myrbäck, K., Eds., *The Enzymes 5* (1961) 367.
4. Weidman, S. W. and Drysdale, G. R. *Fed. Proc. Fed. Am. Soc. Exp. Biol. 32* (1973) 549 Abs.
5. Tabuchi, T. and Uchiyama, H. *Agric. Biol. Chem. 40* (1976) 1411.
6. Hanson, K. R. and Rose, I. A. *Proc. Natl. Acad. Sci. U.S.A. 50* (1963) 981.
7. Gottschalk, G. and Barker, H. A. *Biochemistry 5* (1966) 1125.
8. Gottschalk, G. and Barker, H. A. *Biochemistry 6* (1967) 1027.
9. Srere, P. A., Matsuoka, Y. and Mukherjee, A. *J. Biol. Chem. 248* (1973) 8031.
10. Johansson, C. J., Måhlén, A. and Pettersson, G. *Biochim. Biophys. Acta 309* (1973) 466.
11. Weidman, S. W., Drysdale, G. R. and Mildvan, A. S. *Biochemistry 12* (1973) 1874.
12. Srere, P. A. *J. Biol. Chem. 241* (1966) 2157.
13. Johansson, C. J. and Pettersson, G. *Eur. J. Biochem. 42* (1974) 383.
14. Måhlén, A. *FEBS Lett. 51* (1975) 294.
15. Carrell, H. L., Glusker, J. P., Villafranca, J. J., Mildvan, A. S., Dummel, R. J. and Kun, E. *Science 170* (1970) 1412.
16. Stadtman, E. R. *Methods Enzymol. 3* (1957) 931.
17. Srere, P. A., Brazil, H. and Gonen, L. *Acta Chem. Scand. 17* (1963) S129.
18. Yankeelov, J. A. *Methods Enzymol. 25* (1972) 566.

Received May 23, 1977.

Reduction of 3,3,5,5-Tetramethylcyclopentane-1,2-dione and its Reaction with Methylmagnesium Bromide

TAPIO SIMONEN and TARJA LAITALAINEN

Department of Chemistry, University of Helsinki, SF-00100 Helsinki 10, Finland

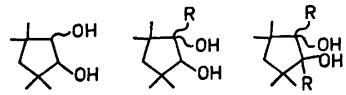
In connection with our interest in reactions [1] of 3,3,5,5-tetramethylcyclopentane-1,2-dione [2] and especially the equilibria [3] in alkaline solutions we found it desirable to synthesize a series of cyclopentane-1,2-diols (Scheme 1).

We report here the preparations of *cis*- and *trans*-3,3,5,5-tetramethylcyclopentane-1,2-diols by reduction of 3,3,5,5-tetramethylcyclopentane-1,2-dione and the spectrometric [4–11] determination of configurations of these new diols. We also describe the reaction of the title compound with methylmagnesium bromide.

Preparation of 3,3,5,5-tetramethylcyclopentane-1,2-diols. When 3,3,5,5-tetramethylcyclopentane-1,2-dione was reduced with sodium borohydride in methanol at room temperature, the only isolated product was *trans*-3,3,5,5-tetramethylcyclopentane-1,2-diol (*trans-1*). The crystallized product showed only one peak analyzed by glass capillary gas chromatography.

The diketone was hydrogenated at about 100 atm. and 60 – 70 °C to give exclusively the diol *cis-1*. Hydrogenation at lower temperatures was incomplete.

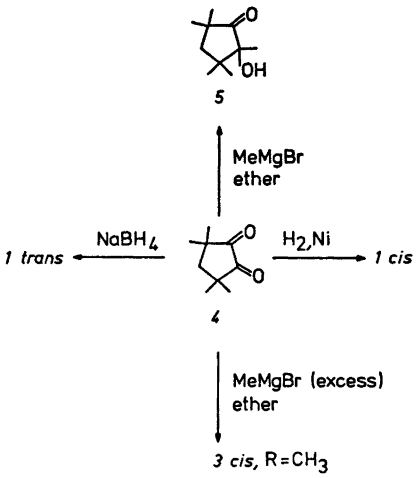
Determination of configurations. In some cases it is possible to assign the configurations by comparing the IR spectra of the isomers in the OH stretching region.[5,6,12,13] In the case of cyclopentane-1,2-diols only the *cis*-isomer is capable of forming an intramolecular hydrogen bond thus showing both the absorptions of free and bonded OH groups in dilute (< 0.005 M) solutions.



1 *2* *3*

The IR spectra of *cis-1* and *trans-1* were measured by using 0.005 and 0.0025 M CCl_4 solutions. The *cis*-isomer shows two OH bands at 3643 (free) and 3576 cm^{-1} (bonded) the intensity ratios being equal in both concentrations.

The spectrum of *trans-1* shows only one OH band (3630 cm^{-1}). When the concentration is 0.005 M, the *trans*-isomer also shows a band at *ca.* 3400 cm^{-1} due to an intermolecular hydrogen bond, which does not exist at higher dilution (0.0025 M).



The ^1H NMR spectra of the diols show characteristic differences. The methylene protons of *cis-1* and *trans-1* give an AB and an A_2 spectrum, respectively. The methine proton in the *cis*-isomer absorbs at a lower field than in the *trans*-isomer. This is in accordance with earlier findings.[9] The absorption signals of all the 12 methyl protons in *cis-1* are observed as one singlet, but the ^{13}C shielding differences of the corresponding carbons are clear (see Experimental). We found an analogous result in the case of *cis-3*.

Finally, *cis-1* and *trans-1* can be distinguished by studying ^{13}C NMR spectra. The differences in ^{13}C shieldings are most striking in the cyclopentane ring itself and especially at the nonprotonated carbons.

The results show that in the case of symmetrically 3,3,5,5-substituted cyclopentane-1,2-diols, the ^1H NMR method presented is applicable for assignment of the configuration even if only one of the isomers is available.

Reaction of 3,3,5,5-tetramethylcyclopentane-1,2-dione with methylmagnesium bromide. The title compound reacts with an equimolar amount of the Grignard reagent yielding after hydrolysis 2-hydroxy-2,3,3,5,5-pentamethylcyclopentanone. The other carbonyl group remains unattacked when using a small excess of magnesium (17 %). No impurities were observed by glass capillary GLC. The structure was established with ^{13}C NMR, ^1H NMR, mass, IR and UV spectra.

We found that 2,2,4,4-tetramethylcyclopentanone is much less reactive toward the same Grignard reagent than the corresponding diketone. Under the same reaction conditions (17 % excess of magnesium) only about 50 % of the starting monoketone reacted. When the product from this preparation was treated with a 3-fold excess of the Grignard reagent, the reaction was still incomplete.

The greater reactivity of one carbonyl group of the diketone compared with the corresponding monoketone may be accounted for by the planar conformation [14] of the ring and nearly *cis*-coplanar carbonyl groups [15] of the former compound. The monoketone and the product *5* have energetically more favoured half-chair conformations.[16]

cis-1,2,3,3,5,5-Hexamethylcyclopentane-1,2-diol was synthesized starting from the title compound and using the 2-step method described above. The configuration was found to be *cis* by using the spectrometric method described in this paper (see Experimental). Analogous results have been observed in the reaction of 3,3,4,4-tetramethylcyclobutane-1,2-dione with methylmagnesium iodide.[17]

Experimental. ^1H NMR spectra were obtained with a JEOL JNM-PMX60 NMR spectrometer and ^{13}C NMR spectra with a JEOL JNM PFT100 spectrometer. IR spectra were recorded with a Perkin-Elmer 125 Grating Spectrometer in 10 % CCl_4 solutions and (NIR) in 0.005 and 0.0025 M solutions using the cell length of 1.0 cm. UV spectra were measured with a Shimadzu UV-200 Double Beam Spectrometer. Mass spectra were recorded with a JEOL JMS-D 100 instrument.

Melting points, determined in open capillary tubes with an electrothermal apparatus, are uncorrected.

3,3,5,5-Tetramethylcyclopentane-1,2-dione (*4*) was synthesized and *methylmagnesium bromide* was prepared as described in Ref. 2.

cis-*3,3,5,5-Tetramethylcyclopentane-1,2-diol* (*cis-1*). The diketone *4* (1.54 g) was hydrogenated in ethanol (70 ml) over Raney nickel at 70 °C using a hydrogen pressure of about 100 atm. When the reaction time was *ca.* 10 h, the orange colour of the diketone had disappeared. The solvent was evaporated in vacuum. The remaining crude product was sublimed (10 mmHg/water bath). The yield of the twice sublimed colourless crystals was 1.05 g (66 %), m.p. 58 °C. ^1H NMR (60 MHz, $CDCl_3$): δ 1.03 (12 H, s), 1.50 (centre of AB q, 2 H, J 14 Hz), *ca.* 3.0 (2 H, s, depending on conc.), 3.76 (2 H, s). ^{13}C NMR (25.15 MHz, $CDCl_3$): δ 25.2 (off-res. q), 30.3 (q), 39.5 (s), 53.1 (t), 81.7 (d). NIR (0.0025 M CCl_4): 3643 (free OH), 3576 cm^{-1} (intramolecularly bonded OH). MS (70 eV, *m/e*): 158 (M).

trans-*3,3,5,5-Tetramethylcyclopentane-1,2-diol* (*trans-1*). The diketone *4* (1.0 g) was dissolved in methanol (15 ml). Sodium borohydride (0.34 g) was added in portions in the course of 20 min at room temperature. The mixture was stirred further for 30 min. Most of the solvent was removed by distillation. The remaining mixture was poured into water (30 ml) containing some diluted hydrochloric acid. The aqueous solution was saturated with sodium chloride, extracted with diethyl ether and the ether solution was dried with magnesium sulfate. After removing the solvent the crude

product was recrystallized from aqueous propanol. The yield of the colourless crystals was 0.72 g (70 %), m.p. 102–103 °C. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.93 (6 H, s), 1.08 (6 H, s), 1.48 (2 H, s), ca. 3.0 (2 H, s, depending on conc.), 3.58 (2 H, s). ^{13}C NMR (25.15 MHz, $CDCl_3$): δ 24.8 (off-res. q), 29.9 (q), 35.3 (s), 52.1 (t), 83.5 (d). NIR (0.0025 M CCl_4): 3630 cm^{-1}. MS (70 eV, *m/e*): 158 (M).

2-Hydroxy-2,3,3,5,5-pentamethylcyclopentanone (5). The diketone *4*, (1.54 g) dissolved in dry diethyl ether, was added to a diethyl ether solution of methylmagnesium bromide, prepared from 0.28 g of magnesium turnings, during half an hour. The temperature of the stirred mixture was kept at 15 °C. The addition complex was hydrolysed with cold ammonium chloride solution, and the aqueous solution was then extracted with diethyl ether. The ether solution was dried with calcium sulfate. After removing the solvent the crude product was sublimed under vacuum. The yield of the colourless product was 0.92 g (54 %), m.p. 45–47 °C. ^{1}H NMR (60 MHz, CCl_4): δ 0.93 (3 H, s), 1.02 (3 H, s), 1.12 (3 H, s), 1.14 (3 H, s), 1.17 (3 H, s), 1.70 (centre of AB q, 2 H, J 13 Hz), *ca.* 2.5 (1 H, s, depending on concentration). ^{13}C NMR (25.15 MHz, $CDCl_3$): δ 20.7 (off-res. q), 23.7 (q), 24.1 (q), 27.9 (q), 28.3 (q), 39.7 (s), 40.7 (s), 49.2 (t), 82.0 (s), 225.4 (s). IR (10 % CCl_4): 3500 (OH), 1738 cm^{-1} (C=O). UV [abs. ethanol (ε)]: 307 (41) nm. MS (70 eV, *m/e*): 170 (M).

cis-*1,2,3,3,5,5-Hexamethylcyclopentane-1,2-diol (cis-3).* The diketone *4* (1.54 g) dissolved in dry diethyl ether (7 ml) was added to the Grignard reagent solution prepared from 0.58 g of magnesium turnings during one hour at room temperature. After the addition the reaction mixture was refluxed for 3 h. After a work-up similar to that described above the crude product was dried and was then treated with a 3-fold excess of methylmagnesium bromide. The reaction product was isolated as described above. After removing the solvent the white mass solidified. The crude diol was purified by vacuum sublimation. The yield of the colourless crystals was 0.25 g (13 %), m.p. 58–62 °C. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.97 (6 H, s), 1.12 (12 H, s), 1.52 (centre of AB q, 2 H, J 14 Hz), *ca.* 2.5 (2 H, s, depending on concentration). ^{13}C NMR (25.15 MHz, $CDCl_3$): δ 14.7 (off-res. q), 21.4 (q), 26.8 (q), 41.3 (s), 54.8 (t), 83.7 (s). IR (10 % CCl_4): 3625, 3533, 3435 cm^{-1} (OH). NIR (0.0025 and 0.005 M CCl_4): 3627 (free), 3560 cm^{-1} (intramolecularly bonded), $\Delta\nu$ 67 cm^{-1}. MS (70 eV, *m/e*): 186 (M).

Each of the new compounds (*cis-1*, *trans-1*, *cis-3*, *5*) showed only one peak by glass capillary GLC using Carlo Erba Fractovap Mod. G I and FFAP stationary phase in capillary column (20 m × 0.3 mm ID).

Acknowledgements. The financial support of the E. Aaltonen Foundation is gratefully acknowledged (T.L.). The authors wish to thank Matti Karhu, Phil.Lic. for technical assistance in catalytic hydrogenations, Antti Hesso, M.Sc. for preparing the glass capillary column, Heikki Pyysalo, Ph.D. (Technical Research Centre of Finland) for running the mass spectra, Mrs. Eila Bernström, M.Sc. for recording the IR spectra and Miss Liisa Heino for obtaining the ^{13}C NMR spectra.

1. Simonen, T. *To be published.*
2. Simonen, T. and Laitalainen, T. *Finn. Chem. Lett.* (1977) 144.
3. Simonen, T. and Hirsjärvi, P. *Finn. Chem. Lett.* (1975) 59.
4. Tichy, M. *Adv. Org. Chem. 5* (1965) 115.
5. Kuhn, L. P. *J. Am. Chem. Soc. 74* (1952) 2492.
6. Kuhn, L. P. *J. Am. Chem. Soc. 76* (1954) 4323.
7. Winkler, J. and McLafferty, F. W. *Tetrahedron 30* (1974) 2971.
8. Fujise, S., Hazato, G., Ikenoue, T. and Sasaki, S. *Bull. Chem. Soc. Jpn. 35* (1962) 1742.
9. Gianni, M., Saavedra, J., Myhalyk, R. and Wursthorn, K. *J. Phys. Chem. 74* (1970) 210.
10. Yonezawa, T., Saito, H., Matsuoka, S. and Fukui, K. *Bull. Chem. Soc. Jpn. 38* (1965) 1431.
11. Anet, F. A. L. *Can. J. Chem. 39* (1961) 789.
12. Takeshita, T. and Kitajima, M. *Bull. Chem. Soc. Jpn. 32* (1959) 985.
13. Angyal, S. J. and Young, R. J. *J. Am. Chem. Soc. 81* (1959) 5467.
14. Goaman, L. C. G. and Grant, D. F. *Tetrahedron 19* (1963) 1531.
15. Leonard, N. J. and Mader, P. M. *J. Am. Chem. Soc. 72* (1950) 5388.
16. McQuillin, F. J. *Alicyclic Chemistry*, William Clowes & Sons, London, Beccles and Colchester 1972, p. 25.
17. Barnier, J.-P. and Conia, J.-M. *Bull. Soc. Chim. Fr.* (1976) 281.

Received March 28, 1977.