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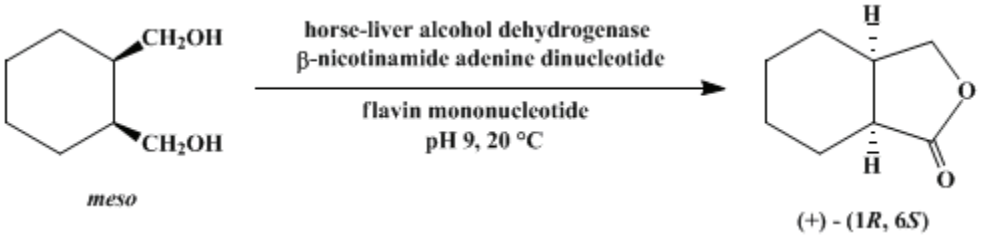
PREPARATION OF CHIRAL, NONRACEMIC γ-LACTONES BY ENZYMECATALYZED OXIDATION OF *meso*-DIOLS: (+)-(1*R*,6*S*)-8-OXABICYCLO[4.3.0]NONAN-7-ONE

[1(3*H*)-Isobenzofuranone, hexahydro-, (3*aS*-*cis*)-]



meso

(+) - (1*R*, 6*S*)

Submitted by J. Bryan Jones and Ignac J. Jakovac[1].
Checked by Roland H. Weber, Max F. Züger, and Dieter Seebach.

1. Procedure

In a 1-L Erlenmeyer flask are placed 475 mL of distilled water (Note 1) and 3.75 g (0.05 mol) of reagent-grade glycine, and the pH is adjusted to 9 by the careful addition of aqueous 10% sodium hydroxide. In the buffer solution thus obtained are dissolved 2.00 g (13.87 mmol) of *cis*-1,2-bis (hydroxymethyl) cyclohexane (Note 2), 0.58 g (0.852 mmol) of β-NAD (Note 3), and 7.8 g (16.2 mmol) of FMN (Note 4). To the clear orange solution obtained is added 80 units of horse-liver alcohol dehydrogenase (Note 5). After the solution is gently swirled for 1 min, the pH is readjusted to 9 and the mixture is kept at room temperature (Note 6) with the mouth of the flask loosely covered by a watchglass. After a few minutes the color of the solution begins to darken and after several hours becomes an opaque green-brown. The pH is readjusted to 9 after 6, 12, 24, 48, and 72 hr by the careful addition of aqueous 10% sodium hydroxide since the pH of the mixture drops progressively as the reaction proceeds. After 4 days (Note 7), the mixture is brought to a pH of ca. 13.3 by the addition of 20 mL of aqueous 50% sodium hydroxide solution. After 1 hr, the mixture is continuously extracted with chloroform for 10 hr (Note 8). The chloroform extract is discarded. The aqueous layer is acidified to pH 3 with concentrated hydrochloric acid and again extracted continuously for 15 hr with chloroform. To the green–orange solution are added charcoal (0.5 g), and magnesium sulfate. The dried and partially decolorized mixture is filtered through a bed of Celite, and the chloroform is removed under reduced pressure using a rotatory evaporator. The residual orange–green oil is distilled in a Kugelrohr apparatus to give 1.4–1.5 g (72–77% yield, (Note 9)) of (+)-(1*R*,6*S*)-8-oxabicyclo[4.3.0]nonan-7-one (>97% e.e., (Note 10)) as a colorless oil, bp 85–100°C (0.1–0.05 mm), mp 26–29°C, $[\alpha]_D^{22}$ + 51.3° ($CHCl_3$, *c* 1.1) (Note 11).

2. Notes

1. It is not necessary to use doubly distilled or deionized water in this buffer preparation.
2. *cis*-1,2-Bis(hydroxymethyl)cyclohexane was purchased from Aldrich Chemical Company, Inc. (or EGA, D-Steinheim).
3. β-NAD is the standard biochemical abbreviation for the coenzyme β-nicotinamide adenine dinucleotide. The β-NAD used was of 95% purity and was purchased from Kyowa Hakko (USA), New York. It is also available from Sigma Chemical Company.
4. FMN is the standard biochemical abbreviation for flavin mononucleotide (or riboflavin phosphate). The sodium salt (95–97% pure) of FMN is used. This grade is inexpensive and is available from Sigma Chemical Company. Its purpose is to effect recycling[2] of the catalytic amount used of the much more costly NAD. A larger than stoichiometric amount of FMN is employed in order to ensure rapid recycling of the NAD.
5. Horse-liver alcohol dehydrogenase (HLADH or LADH, also called "equineliver alcohol

dehydrogenase") is the crystalline preparation (>98% protein) sold by Sigma Chemical Company. It is also available from Worthington and Boehringer. The amount added is quoted in units of activity since the activity of the enzyme from different sources can vary. For example, the Sigma enzyme is sold as having an activity of 1–2 units per milligram of protein. The enzyme used in this preparation had 1.5 units of activity per milligram. We have used Worthington–Boehringer enzyme with equal success. The activity of the enzyme diminishes slowly on prolonged storage, even at −20°C. For controlled results, the enzymatic activity may be determined prior to use and the requisite number of units used.

The assay method of Dalziel[3] is convenient. In a recording UV spectrophotometer set at 340 nm is placed a 3-mL quartz cuvette containing 2.4 mL of 0.10 *M* glycine–sodium hydroxide buffer solution, pH 9, 500 µL of a 54 m*M* solution of ethanol in the same buffer, and 100 µL of a 15 m*M* solution of NAD, also in the same pH 9 buffer. The volume is made up to 3.0 mL, and the assay initiated by the addition of 10 µL of a 1 mg/mL solution of HLADH in 0.10 M "Trishydrochloric acid buffer," pH 7.4. The change in optical density at 340 nm is monitored at 25°C and the activity calculated from the following equation:

$$\text{Units of activity/mg protein} = \frac{\Delta OD_{340}/\text{min}}{6.23 \times \text{mg HLADH/mL of assay volume}}$$

If the preceding assay concentrations are followed exactly, this becomes:

$$\text{Units/mg protein} = \frac{\Delta OD_{340}}{\text{min}/20.75}$$

6. Ambient temperatures of up to 30°C can be employed but the reaction temperature should not be allowed to fall below 20°C.

7. The end of the reaction is checked by gas chromatography using 3% QF-1 or OV-101 on Chromosorb columns. The checkers used an OV-101, at 190°C oven temperature. A sample is extracted with ether. The organic layer is analyzed. At 20°C the reaction usually goes to completion within 4 days.

8. This removes residual starting material and other nonacidic impurities.

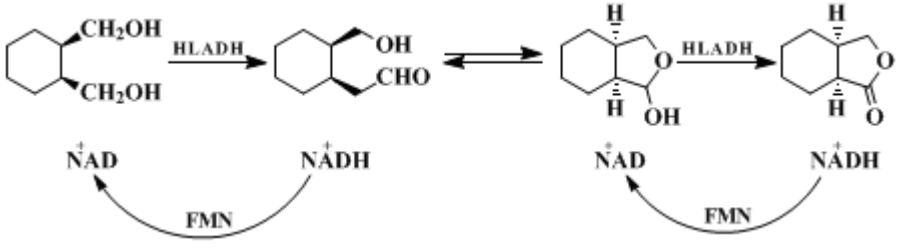
9. Scaling up the preparation is easily accomplished. It is best done by increasing the number of reaction vessels rather than by increasing the reaction volume. For example, 10 g of the *cis*-diol substrate can be oxidized simultaneously using 2.5 g in each of four 1-L Erlenmeyer flasks as described in the procedure described above. After 4 days, the reaction mixtures are combined prior to the chloroform extraction and the lactone is isolated.

10. The absolute configuration and optical purity of the lactone was established by its hydrolysis and epimerization to (1*R*,2*R*)-*trans*-2-hydroxymethylcyclohexanecarboxylic acid followed by lithium aluminum hydride reduction to (1*R*,2R)-*trans*-1,2-bis(hydroxymethyl)cyclohexane.[4] By ^{1}H NMR,[5] the e.e. was >97%.

11. The spectral properties of the product obtained were as follows: IR (thin film): C=O at 1770 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.9–2.8 (m, 10 H, all cyclohexane H), 3.87–4.34 (m, 2 H, CH_2-O).

3. Discussion

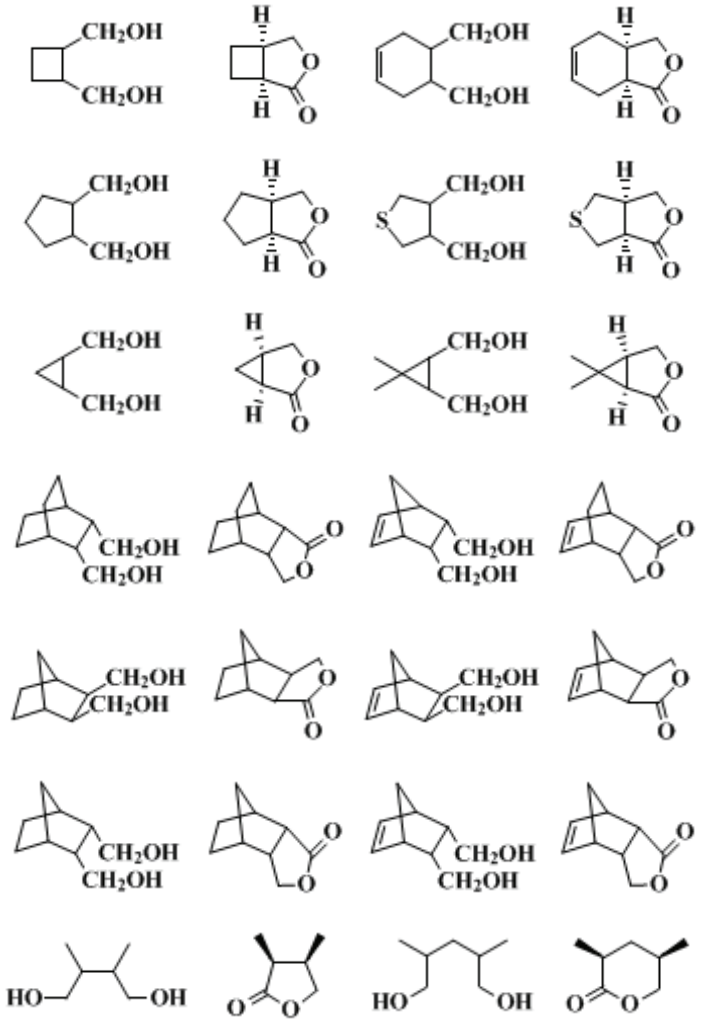
Horse-liver alcohol degydrogenase is a well-documented enzyme capable of operating with high stereoselectivity on a broad structural range of alcohol and carbonyl substrates.[6] The present reaction proceeds via the pathway shown below, where NAD and NADH represent the oxidized and reduced forms, respectively, of the nicotinamide adenine dinucleotide coenzyme.



Chemical oxidations of diols to racemic lactones can be achieved by a broad spectrum of oxidizing agents.[7] At the present time, however, only the enzymatic route described can provide a versatile, one-step, access to such a wide range of highly enantiomerically enriched γ-lactones, useful as chiral building blocks for syntheses.

The lactones thus far obtained by this route have been assembled in Table I. Each oxidation proceeds in high chemical yield (65–90%) to give products of >97% enantiomeric excess.[5]

TABLE I
PREPARATION OF γ-LACTONES BY HLADH-CATALYZED OXIDATIONS OF *meso*-DIOLS (YIELD[ref.])[a]



[a] The optical purities and/or enantiomeric excesses were determined by ^{1}H NMR to be >97%;[5] **2** was obtained with 85% e.e.

The maximum reaction time required for any one of the substrates shown in Table I is 7 days. In reaction mixtures that contain lactones **4** and **5**, minor amounts of the hemiacetal intermediates are present; they are removed during the extraction at pH 13. After chromatographic separation from any unreacted diols, they can be readily converted to the corresponding lactones by chemical oxidation with silver carbonate on Celite.[8]

The lactones shown in the Table include several representatives of recognized or potential value as starting materials in natural product synthesis. Lactone **1** is a precursor of grandisol,[9,10] lactone **3** of some pyrethroids,[9,11] lactone **6** of some prostaglandins,[9,12] and lactone **7** of multistriatin,[13] methynolide,[14] and monensin.[15]

This preparation is referenced from:

- Org. Syn. Coll. Vol. 8, 332

[16]

References and Notes

1. Department of Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A1.
2. Jones, J. B.; Taylor, K. E. *Can. J. Chem*. **1976**, *54*, 2969–2973.
3. Dalziel, K. *Acta Chem. Scand*. **1957**, *11*, 397–398.
4. Goodbrand, H. B.; Jones, J. B. *J. Chem. Soc., Chem. Commun*. **1977**, 469–470.
5. Jakovac, I. J.; Jones, J. B. *J. Org. Chem*. **1979**, *44*, 2165–2168.
6. Jones, J. B.; Beck, J. F. *Tech. Chem. (NY)* **1976**, *10*, 107–401.
7. Kano, S.; Shibuya, S.; Ebata, T. *Heterocycles* **1980**, *14*, 661–711.
8. Fétizon, M.; Golfier, M.; Louis, J.-M. *J. Chem. Soc., Chem. Commun*. **1969**, 1118–1119; Fétizon, M.; Golfier, M.; Louis, J.-M. *Tetrahedron* **1975**, *31*, 171–176.
9. Jakovac, I. J.; Ph.D. Thesis, University of Toronto, **1980**; Jones, J. B.; Finch, M. A. W.; Jakovac, I. J. *Can. J. Chem*. **1982**, *60*, 2007–2011.
10. Katzenellenbogen, J. A. *Science* **1976**, *194*, 139–148.
11. Elliott, M.; Janes, N. F. *Chem. Soc. Rev*. **1978**, *7*, 473–505.
12. Jones, G.; Raphael, R. A.; Wright, S. *J. Chem. Soc., Perkin Trans. I* **1974**, 1676–1683.
13. Bartlett, P. A.; Myerson, J. *J. Org. Chem*. **1979**, *44*, 1625–1627.
14. Nakano, A.; Takimoto, S.; Inanaga, J.; Katsuki, T.; Ouchida, S.; Inoue, K.; Aiga, M.; Okukado, N.; Yamaguchi, M. *Chem. Lett*. **1979**, 1019–1020.
15. Collum, D. B.; McDonald, III, J. H.; Still, W. C. *J. Am. Chem. Soc*. **1980**, *102*, 2118–2120.
16. Jakovac, I. J.; Ng, G.; Lok, K. P.; Jones, J. B. *J. Chem. Soc., Chem. Commun*. **1980**, 515–516; Jakovac, I. J.; Goodbrand, H. B.; Lok, K. P.; Jones, J. B. *J. Am. Chem. Soc*. **1982**, *104*, 4659–4665.

Appendix
Chemical Abstracts Nomenclature (Collective Index Number); (Registry Number)

nicotinamide adenine dinucleotide coenzyme

ethanol (64-17-5)

hydrochloric acid (7647-01-0)

ether (60-29-7)

sodium hydroxide (1310-73-2)

chloroform (67-66-3)

Glycine (513-29-1)

magnesium sulfate (7487-88-9)

lithium aluminum hydride (16853-85-3)

silver carbonate (534-16-7)

cis-1,2-bis(hydroxymethyl) cyclohexane,
cis-1,2-Bis(hydroxymethyl)cyclohexane (5059-76-7)

(+)-(1R,6S)-8-OXABICYCLO[4.3.0]NONAN-7-ONE

(1R,2R)-trans-2-hydroxymethylcyclohexanecarboxylic acid

(1R,2R)-trans-1,2-bis(hydroxymethyl)cyclohexane

1(3H)-Isobenzofuranone, hexahydro-, (3aS-cis)- (65376-02-5)