BIOORGANIC CHEMISTRY 13, 131–134 (1985)

Absolute Stereochemistry of (+)-*trans*-1,2-Dihydroxyacenaphthene, a Mammalian Metabolite of Acenaphthylene

MITSURU IMUTA,[1] MASAJI KASAI,[2] AND HERMAN ZIFFER[3]

Laboratory of Chemical Physics, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205

Received October 11, 1984

A sample of (+)-*trans*-1,2-dihydroxyacenaphthene, a mammalian metabolite of acenaphthylene, was prepared by stereoselective partial hydrolysis of the corresponding synthetic racemic diacetate using the mold, *Rhizopus nigricans*. The absolute stereochemistry of the *trans*-diol was established as (1*R*,2*R*) by conversion to the known dimethyl (2*S*,3*S*)diacetoxysuccinate.

The usual oxidative metabolic route for an aromatic hydrocarbon in mammals has been shown to proceed through initial oxidation of the substrate by a cytochrome *P*-450 enzyme to an arene oxide, which is then enzymatically hydrated (by hydrolase) to a *trans*-dihydrodiol (*1*). However, the *in vivo* metabolism of two aromatic hydrocarbons, indene and acenaphthylene, appears to follow a different sequence, in that rats and rabbits fed these compounds excrete mixtures of both *cis*- and *trans*-diols. Since the absolute stereochemistry of these metabolites had not been determined and since the metabolism of these substrates appeared to differ from that of other aromatic hydrocarbons, we have determined this absolute stereochemistry. Initially we prepared chiral samples of (+)-*cis*- and (+)-*trans*-1,2-dihydroxyindane (*2*) and related these compounds to 1*R*-indanol. We now wish to describe the preparation of (+)-*trans*-1,2-dihydroxyacenaphthene, **1a**, the reported metabolite of acenaphthylene (*3*) and the proof of its absolute stereochemistry through chemical transformation to the known dimethyl (2*S*,3*S*)diacetoxysuccinate.

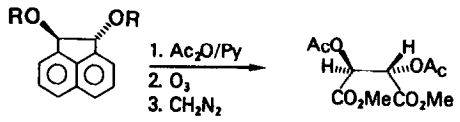
The major obstacle is assigning the absolute stereochemistry of mammalian metabolites has been the limited quantity of material available from enzymic studies. We recently developed a microbially based procedure (*4*, *5*) which employs the enantioselective hydrolysis of racemic esters (acetates) using the mold,

[1] Present address: Shionogi Research Laboratory, Shionogi and Co., Ltd., Fukushima-Ku, Osaka-553, Japan.

[2] Present address: Kyowa Hakko Kogyo Co., Ltd., Tokyo Research Laboratory, 3-6-6 Asahimachi, Machidashi, Tokyo 194, Japan.

[3] To whom correspondence should be addressed.

0045-2068/85 $3.00



1

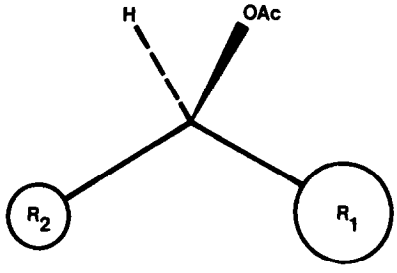
(a) R = H

(b) R = Ac

3

SCHEME 1

Rhizopus nigricans, for the preparation of chiral alcohols and diols. We have now made use of this procedure to obtain a sample of (+)-**1a**. In addition to serving as a valuable procedure for the synthesis of chiral compounds, this method can be used, as described below, to predict the absolute stereochemistry of the alcohol formed in conjunction with a rule, which states that the enantiomer shown as **2** is the one more rapidly hydrolyzed, where R_1 is larger than R_2. Since there are no data available on the relative sizes of a fused 1,8-naphthalene system and a substituted saturated carbon atom in a cyclic carbinol, the absolute stereochemistry of the diol formed in this case cannot be predicted. However, in earlier work it was shown that a fused benzene ring is effectively smaller than a substituted vicinal carbon; one might therefore assume the same relative sizes of the aromatic moieties and a substituted saturated carbon.



2

The absolute stereochemistry of (+)-**1a** formed by the partial hydrolysis of the racemic diacetate was established as shown in Scheme 1. The diol was first acetylated and then ozonized (exhaustively) to destroy the aromatic rings. The mixture of acids obtained from an oxidative workup was methylated with diazomethane, and a sample of (+)-**3** was isolated and purified by chromatography. An authentic sample of the (+) enantiomer had previously been prepared (*6*) from (−)-tartaric acid of known absolute stereochemistry. These chemical transformations thus establish the absolute stereochemistry of (+)-**1a** as 1*R*,2*R*. Since Hopkins *et al.* (*3*) isolated the (+) enantiomer as a metabolite of acenaphthylene, the metabolite has a 1*R*,2*R* configuration[4].

[4] The absolute stereochemistry of the (+)-diol was reported (*7*) as 1*R*,2*R* in abstracts of a meeting by Nakazaki *et al.*; however, no publication of the work has appeared. The chirality of the dibenzoate of the (+)-diol is shown as negative by Nakanishi (*8*). The absolute stereochemistry was assigned using the dibenzoate chirality rule; however, Nakanishi (*8*) indicates possible problems because of the presence and proximity of the dibenzoate and naphthalene chromophores.

EXPERIMENTAL PROCEDURES

Melting points were determined on a hot-stage apparatus; they are uncorrected. Proton magnetic resonance spectra were recorded on a Varian HR-220 MHz instrument, and optical rotations on a Perkin–Elmer 241 MC polarimeter. Preparative and analytical TLC work was performed on plates coated with silica gel F-254.

Preparation of (±)-*trans-1,2-diacetoxyacenaphthene*. *trans*-1,2-Dihydroxyacenaphthene was prepared as described by Hopkins *et al.* (*3*) through the reaction of acenaphthylene with iodine and silver benzoate, followed by basic hydrolysis (KOH in methanol–water) of the dibenzoate, to yield the *trans*-diol, mp 157–158°C. The diol was acetylated with acetic anhydride and pyridine in the usual manner to yield the diacetate, a solid, mp 44–45°C; ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 6H), 6.55 (s, 2H), 7.27–7.86 (m, 6H).

Microbial hydrolysis. A solution of (±)-*trans*-1,2-diacetoxyacenaphthene (100 mg) in distilled THF (1 ml) was added to a 1-liter Erlenmeyer flask containing a 5-day culture of *R. nigricans* (*4, 5*). The mixture was shaken for 16 h and extracted with ethyl acetate (3×, 250-ml portions). The combined extracts were concentrated and the mixture was separated by thick-layer chromatography (silica gel, 2 mm; 20 × 20-cm plate) using ethyl acetate–hexane (3 : 7) to yield recovered *trans*-1,2-diacetoxyacenaphthene, 53 mg, pale yellow oil, $[\alpha]_D^{25°C}$ +38° (c 0.53 ethanol) and *trans*-1,2-dihydroxyacenaphthene, 23 mg, mp 158–159°C $[\alpha]_D^{25°C}$ +33.2 (c 0.28, $CHCl_3$), $[\alpha]_{546}^{25°C}$ +41.4°; reported (*3*) $[\alpha]_{546}^{25°C}$ +66°.

Ozonolysis of (−)-*trans-1,2-diacetoxyacenaphthene* (**1b**). Acetylation of (+)-*trans*-1,2-dihydroxyacenaphthene with acetic anhydride in pyridine in the usual manner yielded the diacetate, **1b**, as a pale yellow oil, $[\alpha]_D^{25°C}$ −89.1° (c 0.70, EtOH).

A solution of the diacetate (135 mg) in acetic acid–dichloromethane (50 ml, 1 : 1) was ozonized at 0°C using a stream of ozone (2–4%) from an Ozonator, Model 03V2. After 8 h, 2 ml 30% hydrogen peroxide was added and the reaction mixture was stirred at 50°C for 0.5 h. Excess hydrogen peroxide was decomposed with sodium sulfite and the solvent was removed *in vacuo*. The residue was treated with excess aqueous sodium bicarbonate and the solution was extracted with hexane. The aqueous layer was acidified, saturated with sodium chloride, and extracted several times with ethyl acetate. The organic layer was dried and concentrated. The residue was dissolved in ether and treated with a freshly prepared diazomethane solution. The ether solution was concentrated and the residue was distilled *in vacuo* (97–99°C/0.2 Torr) to yield a colorless oil, which was crystallized from hexane to give a pure sample of (+)-dimethyl-(2*S*,3*S*)diacetoxysuccinate; 19 mg, mp 102–3°; $[\alpha]_D^{25°C}$ +11.9° (c 1.8, $CHCl_3$). The e.e. was calculated as 50% based on the specific rotation of an authentic sample previously prepared (*6*) from (−)-tartaric acid, $[\alpha]_D^{25°C}$ +23.7° (c 1.52, $CHCl_3$).

REFERENCES

1. THAKKER, D. R., YAGI, H., LEVIN, W., WOOD, A. W., CONNEY, A. H., AND JERINA, D. M. (1985) *in* Bioactivation of Foreign Compounds (Anders, M. W., Ed.), pp. 177–241, Academic Press, New York.

2. (a) IMUTA, M., AND ZIFFER, H. (1978) *J. Org. Chem.* **43,** 4540; (b) BOYD, D. R., SHARMA, N. D., AND SMITH, A. E. (1982) *J. Chem. Soc., Perkin Trans. 1,* 2767.
3. HOPKINS, R. P., BROOKS, C. J. W., AND YOUNG, L. (1962) *Biochem. J.* **82,** 457.
4. ZIFFER, H., KAWAI, K., KASAI, M., IMUTA, M., AND FROUSSIOS, C. (1983) *J. Org. Chem.* **48,** 3017.
5. KASAI, M., KAWAI, K., IMUTA, M., AND ZIFFER, H. (1984) *J. Org. Chem.* **49,** 675.
6. IMUTA, M., AND ZIFFER, H. (1978) *J. Org. Chem.* **43,** 3319.
7. MIURA, R., OKADA, J., OHNO, T., AND NAKAZAKI, M. (1971) *in* "Proceedings, 15th Symposium on the Chemistry of Natural Products, Nagoya, Japan, 1971," p. 69.
8. HARADA, N., AND NAKANISHI, K. (1983) Circular Dichroic Spectroscopy, Exciton Coupling in Organic Stereochemistry, p. 155, University Science Books,