

Conformational Studies of 3-Hydroxy- α -damascone by Nuclear Overhauser Effect Nuclear Magnetic Resonance Experiments

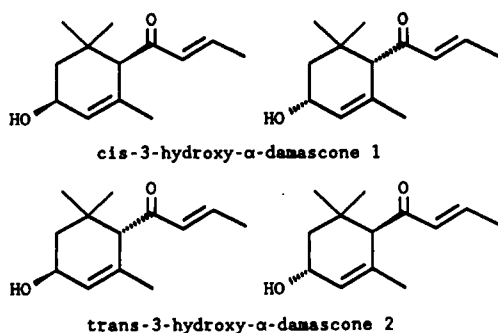
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A series of NOE experiments was carried out to clarify the conformation of *cis*- (1) and *trans*-3-hydroxy- α -damascone (2). For 1, half-chair conformation with a quasiequatorial hydroxy group and a quasixial side chain was determined. A main half-chair conformation with quasiequatorial positions both for the hydroxy group and for the side chain was also found for 2. According to the NOE experiments, an additional boat conformation with an equatorial hydroxy group and an axial side chain was postulated for 2.

INTRODUCTION

Recently, *cis*- (1) and *trans*-3-hydroxy- α -damascone (2) have been identified as microbial bioconversion products of α -damascone (Schoch et al., 1991), an α -ionone isomer



widely used in the flavor and fragrance industry. A synthetic route leading to a mixture of 1 and 2 has already been described (Takei et al., 1973), but spectroscopic differentiation of the diastereomers has not been carried out as yet. This paper concerns conformational studies of 1 and 2 using NOE NMR experiments.

EXPERIMENTAL PROCEDURES

3-Hydroxy- α -damascone. The synthesis of the diastereomers 1 and 2 was performed by bromination of α -damascone with *N*-bromosuccinimide followed by substitution of the Br with OH⁻ by stirring the bromo compounds in 10% Na₂CO₃ solution for 2 days. To isolate the product, LC prefractionation on silica gel (2 × 50 cm glass column, Kieselgel 60; Merck, Darmstadt; 35 mL/min) with pentane-diethyl ether (1:1) was performed. Finally, preparative HPLC on a silica gel column (16 × 250 mm, 5- μ m particle size; Knauer, Berlin; 10 mL/min) using hexane-*tert*-butyl methyl ether (1:1) as eluent led to the separation of 1 and 2. Their chromatographic and spectroscopic data have been published elsewhere (Schoch et al., 1991).

Nuclear Magnetic Resonance. The ¹H NMR spectra were recorded on a Bruker WM 400 spectrometer (400.13 MHz) using benzene-*d*₆ and CDCl₃ as solvents (Me₄Si as internal standard). Using an automated technique, nuclear Overhauser enhancement (NOE) measurements (cf. Figures 1 and 2) of the carefully degassed samples (0.5%) were performed at ambient temperature by irradiation of the different proton chemical shift frequencies for 4.8 s. The relaxation time was 3 s. Before the irradiation frequency was changed, there was a relaxation delay of 15 s.

RESULTS AND DISCUSSION

For the conformational studies of 1 and 2, first of all, the ¹H NMR data of compounds exhibiting similar structures, i.e., *cis*- and *trans*-3-methoxy- α -ionone

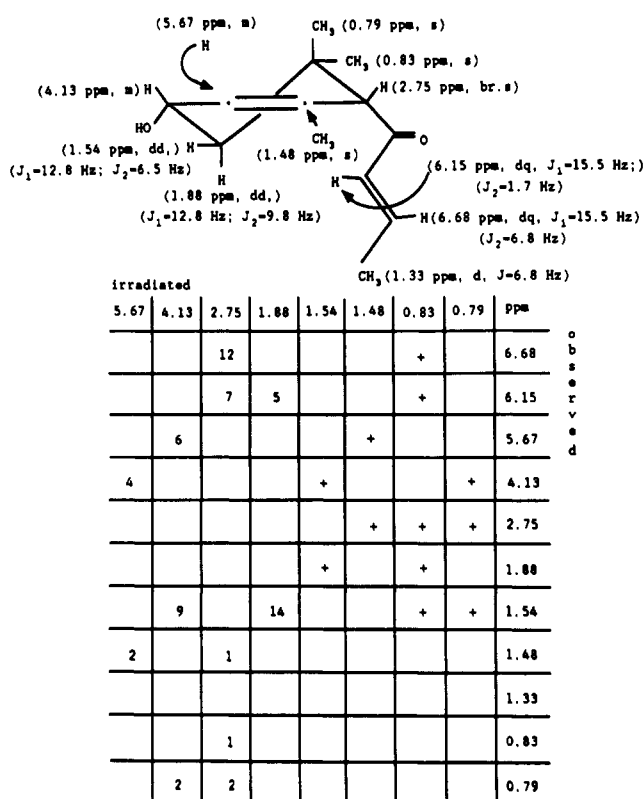


Figure 1. NOE experiments carried out with *cis*-3-hydroxy- α -damascone (1). The horizontal axis demonstrates which signals were irradiated and the vertical axis the signals affected. Values are given in percent except for signals where integration was hampered due to partial irradiation of a second signal (+).

(Buchecker et al., 1974) and carotenoids with an ϵ -end group (Englert, 1981; Mayer, 1981; Foss et al., 1986), were compared with the results obtained in our experiments (Figures 1 and 2). In the cited literature a half-chair conformation has been described for both isomers, i.e., with a quasiequatorial hydroxy group and side chain for the *trans* isomer and a quasiequatorial hydroxy group and a quasixial side chain for the *cis* isomer. The different vicinal coupling constants (δ 1.54 ppm, J = 6.5 Hz; δ 1.88 ppm, J = 9.8 Hz) we observed for the *cis* isomer 1 supported the quasiequatorial position of the hydroxy group. However, the vicinal coupling constants of the *trans* isomer showed nearly identical values (δ 1.40 ppm, J = 5.4 Hz; δ 1.94 ppm, J = 6.0 Hz), contradicting the described quasiequatorial position of the hydroxy group. In previous studies carried out with carotenoids, this discrepancy had also been observed but not discussed (Buchecker et al.,

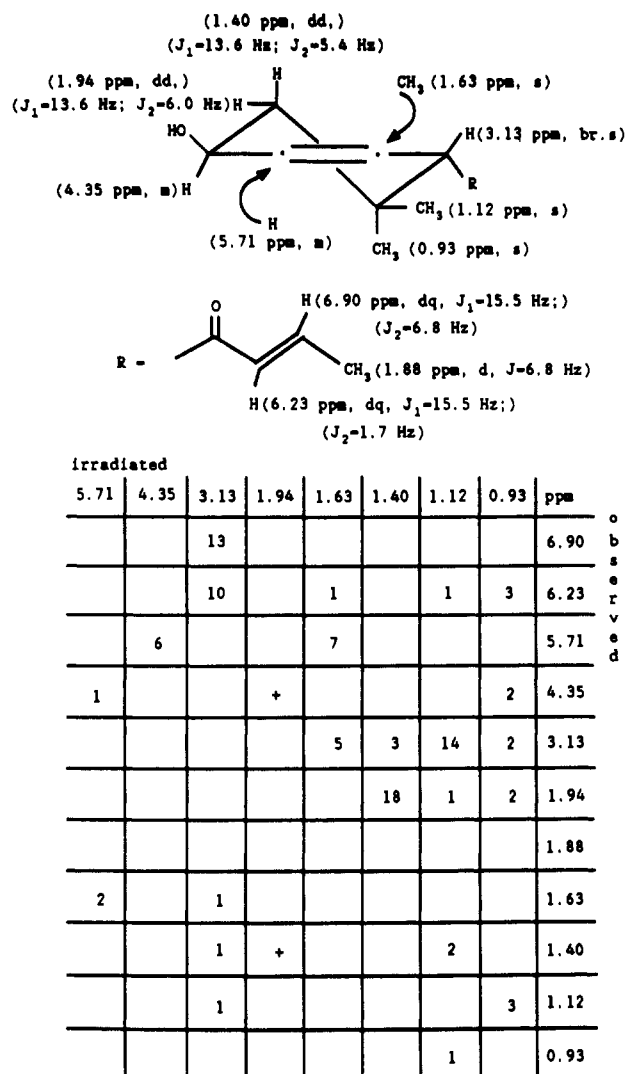


Figure 2. NOE experiments carried out with *trans*-3-hydroxy- α -damascone (**2**). The horizontal axis demonstrates which signals were irradiated and the vertical axis the signals affected. Values are given in percent except for signals where integration was hampered due to partial irradiation of a second signal (+).

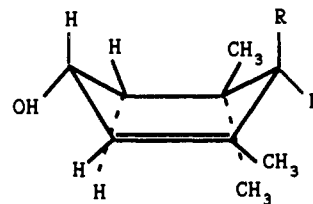
1974; Foss et al., 1986). To clarify the conformation of **1** and **2**, a series of NOE experiments was carried out.

The results obtained with **1** are summarized in Figure 1. Irradiation of the δ 0.79 methyl signal resulted in NOE only for the δ 1.54 proton but not for the δ 1.88 proton, showing the axial position of the δ 0.79 methyl group and the equatorial position of the δ 1.54 proton. By irradiation of the δ 0.79 methyl signal a NOE of the δ 4.13 proton was also observed, indicating a 1,3-diaxial relationship and, consequently, the quasiequatorial position of the hydroxy group. This could be confirmed by irradiation of the δ 1.88 and δ 1.54 protons, because only irradiation of the equatorial δ 1.54 proton caused NOE for the δ 4.13 proton. The quasiequatorial position of the side chain was demonstrated, since irradiation of the δ 1.88 proton resulted in NOE for the δ 6.15 proton, supposing axial positions for both the side chain and the δ 1.88 proton. The quasiequatorial position of the side chain was further confirmed by irradiation of the δ 0.79 and 0.83 methyl signals leading to NOE for the quasiequatorial δ 2.75 proton.

The findings of NOE experiments obtained with **2** are outlined in Figure 2. The evaluation of the data resulted in a main conformation of the *trans* isomer with quasiequatorial positions both for the hydroxy group and for the side chain, corresponding to the data given in the literature (Buchecker et al., 1974; Foss et al., 1986). Irradiation of the δ 0.93 methyl signal resulted in NOE for the δ 1.94, but

not the δ 1.40, proton, showing the axial position of the methyl group and the equatorial position of the δ 1.94 proton. The quasiequatorial position of the hydroxy group and therefore the quasiequatorial position of the δ 4.35 proton were demonstrated, since NOE was obtained upon irradiation of the δ 0.93 methyl signal, requiring an 1,3-diaxial relationship. In addition, only irradiation of the equatorial δ 1.94 proton—and not that of the axial δ 1.40 proton—resulted in NOE for the δ 4.35 proton. The 1,3-diaxial position of the δ 1.40 and 3.13 protons (that corresponds to quasiequatorial positions of the side chain) was evaluated upon irradiation of the δ 3.13 proton leading to NOE at δ 1.40. Irradiation of the δ 1.12 signal resulted in NOE at the proton in the *cis* position at δ 3.13.

However, upon irradiation at the δ 0.93 methyl signal weak NOE at δ 3.13 was also observed. Therefore, the occurrence of a second conformation has to be postulated. As mentioned above, irradiation of the δ 1.40 proton did not lead to NOE for the δ 4.35 proton. Thus, the second half-chair conformation with quasiequatorial position of the side chain and the hydroxy group can be excluded. According to the NOE experiments an additional boat conformation with equatorial hydroxy group and axial side chain is postulated. In this conformation interactions between the side chain and the δ 4.35 proton are possible, leading to a deformation of the dihedral angles. By this effect the above-mentioned unexpected data for the vicinal coupling constants of **2** can be explained.



Finally, it has to be pointed out that attempts to inhibit the ring inversion by low-temperature measurements failed. The temperature of -100 °C obtained in a mixture of acetone- d_6 and $CFCl_3$ (1:1) was not sufficient to record separated signals for both the conformations.

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LITERATURE CITED

- Buchecker, R.; Hamm, P.; Eugster, C. H. Absolute Configuration of Xanthophyll (Lutein). *Helv. Chim. Acta* 1974, 57, 631-656.
- Englert, G. NMR of carotenoids. In *Carotenoid Chemistry and Biochemistry*; Britton, G., Goodwin, T. W., Eds.; Pergamon Press: Oxford, U.K., 1981; pp 107-134.
- Foss, P.; Skjetne, T.; Liaaen-Jensen, S. Algal carotenoids. 36. NMR studies of prasinoxanthin—Stereochemical analysis. *Acta Chem. Scand.* 1986, B40, 172-177.
- Mayer, H. Synthesis of optically active carotenoids with ϵ -end group. In *Carotenoid Chemistry and Biochemistry*; Britton, G., Goodwin, T. W., Eds.; Pergamon Press: Oxford, U.K., 1981; pp 55-70.
- Schoch, E.; Benda, I.; Schreier, P. Bioconversion of α -damascone by *Botrytis cinerea*. *Appl. Environ. Microbiol.* 1991, 57, 15-18.
- Takei, Y.; Mori, K.; Matsui, M. Synthesis of a stereoisomeric mixture of 3-hydroxy- α -damascone. *Agric. Biol. Chem.* 1973, 37, 2927-2928.

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