field than the equatorial counterparts in V. In addition, the H_3 signal of XIII showed higher value, since the *syn*-diaxial relation was not present in this case.

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Masayuki Onda,*1 Masuko Akagawa,*2 Motoko Komamiya, and Fumi Nishiuchi*1 : Concerning the Stereoisomers of Cholest-4-en-3-one 2,4-Dinitrophenylhydrazone.

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Cholest-4-en-3-one 2,4-dinitrophenylhydrazone (2,4-DNPH), which was synthesized from cholest-4-en-3-one[1] under a mild condition to obtain the pure sample for identification, exhibited a wide range of melting point and two colored spots on carefully thin-layer chromatography (TLC). We isolated two compounds by means of preparative TLC; *e.g.* dark red crystal of m.p. 233~234° (decomp.) (D-I) from upper band and red crystal of m.p. 196~197° (decomp.) (D-II) from lower band were respectively obtained. Both compounds were formulated as $C_{33}H_{48}O_4N_4$ by analytical data.

D-I and D-II were converted to cholest-4-en-3-one by hydrolysis,[2] from which a mixture of D-I and D-II was again obtained with 2,4-dinitrophenylhydrazine. On boiling in alcohol or chloroform or benzene for several hours D-I and D-II did not change, but D-II changed to D-I in the presence of a catalytic amount of acetic acid. D-I corresponds to cholest-4-en-3-one 2,4-DNPH, which is known in the literatures,[3,4] from melting point and visible absorption spectra. A possibility that D-II might be cholest-5-en-3-one 2,4-DNPH is discarded from purity of starting material cholest-4-en-3-one[1,4] and visible absorption spectra (Table I). Accordingly, D-I and D-II must be the stereoisomers of cholest-4-en-3-one 2,4-DNPH.

Table I. Visible Absorption Spectra of D-I and D-II

Substance	λ_{max}^{DMF} mμ (ε)	$\lambda_{max}^{CHCl_3}$ mμ (ε)
D-I	404(24,000)	396(32,500)
D-II	396(19,900)	390(31,100)
Cholest-4-en-3-one-2,4-DNPH	405(24,300)a)	
Cholest-5-en-3-one-2,4-DNPH	380(20,000)a)	

a) lit. 4).

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1) L. F. Fieser : J. Am. Chem. Soc., **75**, 5421 (1953).
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It is the accepted fact that there theoretically exist stereoisomers in the compounds containing C=N bond due to restricted rotation around it and, in fact, many oximes were separated into *syn* and *anti* isomers. There is a few case[5] that *syn* and *anti* isomers of 2,4-DNPHs were isolated but in many cases the modern physico-chemical methods[6] revealed that stereoisomers only existed in solutions.

On nuclear magnetic resonance (NMR) studies of aliphatic ketone phenylhydrazones and 2,4-DNPHs Karabatsos, *et al.*[6] found that *cis*-Hα (CH), in which conformation was in the C-C=N plane, resonated at lower fields than *trans*-Hα (CH) and $\Delta\nu$*[3] of *cis*-Hα was much larger than that of *trans*-Hα.

Since the stable comformation of the conjugated system $C_5=C_4H-C_3=N$ of cholest-4-en-3-one 2,4-DNPH is in the same plane, *cis*-C_4-H will be strongly affected to shift for lower field by the anisotropic group than *trans*-C_4-H due to the same reason as is *cis*-Hα(CH) of aliphatic ketone 2,4-DNPH and $\Delta\nu$(*cis*) will be much larger than $\Delta\nu$(*trans*).

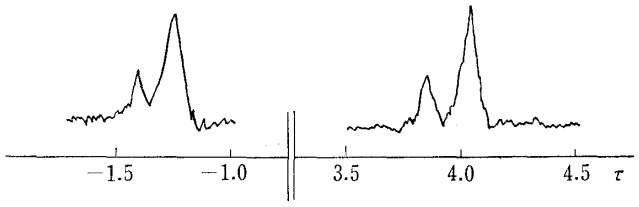

Fig. 1. Nuclear Magnetic Resonance Spectrum of a Mixture of D-I and D-II in $CDCl_3$

As shown in Fig. 1, NMR spectra of a mixture of D-I and D-II reveal difference at two areas (NH and C_4-H). C_4-H appears as two singlets of unequal intensities around 4.0 τ in deuterochloroform, to each of which can be assigned from NMR spectra of pure D-I and D-II. From intensities of each peak the ratio of D-I/D-II is about 3:1. On addition of benzene two singlets are concentrated on one singlet. C_4-H of D-I resonates at upper field than that of D-II and is not affected by benzene, but C_4-H of D-II is strongly affected by benzene (Table II).

Table II. Nuclear Magnetic Resonance Spectra of D-I and D-II

Substance	Solvent	C_4-H	NH	Aromatic-H[a]
A mixture of D-I & D-II	A	3.86, 4.05	−1.40, −1.24	0.91, 1.72, 2.09
	B	4.05	−1.05	1.05, ——, ——
D-I	A	4.05	−1.25	0.91, 1.72, 2.09
	B	4.05	−1.05	1.05, ——, ——
D-II	A	3.86	−1.40	0.91, 1.72, 2.09
	B	4.05	−1.05	1.05, ——, ——

solvent A: $CDCl_3$ solvent B: $CDCl_3$-benzene (1:1 v/v)
a) $J_{3,5}=3.0$ c.p.s., $J_{5,6}=9.8$ c.p.s.

In order to ensure these things we examined NMR spectra of *l*-α-santonin 2,4-DNPH.[7] This compound is considered to be thermodynamically stable form in which C_2-H is *cis* to anisotropic group (Chart 1) and always reveals only one spot on TLC. As shown in Table III, C_2-H is affected by benzene as expected.

Hence, we assign the stable form D-I to *anti* (I) and D-II to *syn* isomer (II) (Chart 2) with fairly reliablity.

Although we now can not comment on the reasons, it is of interest that NH of D-I resonates at upper field than that of D-II and both shift to the same upper field on addition of benzene.

*3 $\Delta\nu=\nu_{benzene}-\nu_{CCl_4 \text{ or } CDCl_3}$

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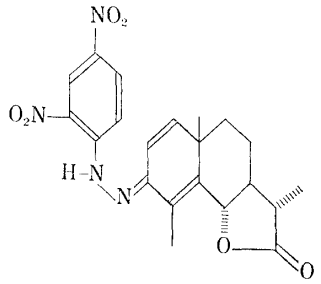

Chart 1.

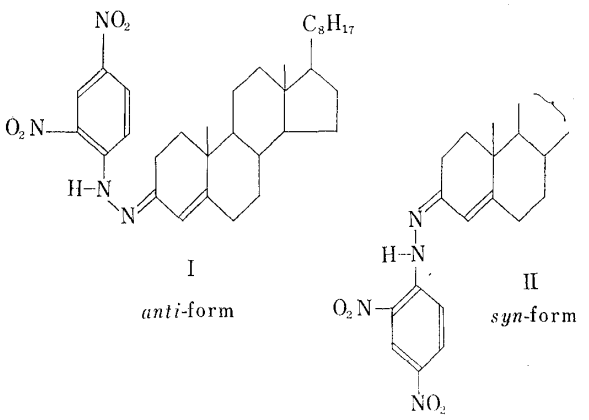

Chart 2.

TABLE III. Nuclear Magnetic Resonance Spectra of *l*-α-Santonin 2,4-Dinitrophenylhydrazone

Solvent	C_2-H	C_1-H	C_4-CH_3
$CDCl_3$	3.65	3.38	7.68
$CDCl_3$-benzene (2:1 v/v)	3.85	3.49	7.68

$J_{1,2}$=10.8 c.p.s., $J_{CH_3,6}$=1.5 c.p.s.

Testosterone acetate 2,4-DNPH, m.p. 218~219° (lit.[8] m.p. 219~220°), which was gained by same method as cholest-4-en-3-one 2,4-DNPH, reveals two spots (Rf 0.31, 0.39*[4]) on TLC. So we are expecting that stereoisomers of many 2,4-DNPHs will be isolated by means of preparative TLC.

Experimental

NMR spectra were measured on a JNM C-60 spectrometer, operating at 60 Mc. with high resolution. The compounds were examined in a 5~10% solution. The chemical shifts were given in τ values and tetramethylsilane was used as an internal standard. Visible absorption spectra were taken with a Hitachi EPS-2U automatic recording spectrometer. All melting points were determined on a Kofler block and uncorrected.

Cholest-4-en-3-one 2,4-DNPH (D-I and D-II)——To a solution of 2,4-dinitrophenylhydrazine (0.3 g.) in EtOH (60 ml.) was added a solution of cholest-4-en-3-one (0.55 g.) in EtOH (1 ml.) and then conc. HCl (0.1 ml.) added. The mixture was allowed to stand at room temperature overnight. The precipitates were filtered and washed with EtOH, giving 0.8 g. of red crystal, m.p. 203~218°(decomp.).

Isolation of D-I and D-II——A solution of the above mixture (0.8 g.) in $CHCl_3$ (5 ml.) was poured through silicagel column (200 g.) treated with benzene and washed with benzene (250 ml.) to discard. It was eluted with benzene (180 ml.) to give fraction-I, whose major component was D-I, and then eluted with $CHCl_3$ (400 ml.) to give fraction-II. Fraction-I afforded 0.38 g. of dark red prisms (D-I), m.p. 233~234° (decomp.) with three times recrystallizations from benzene-EtOH. TLC*[5] Rf 0.70, *Anal.* Calcd. for $C_{33}H_{48}O_4N_4$: C, 70.18; H, 8.57; N, 9.92. Found: C, 70.05; H, 8.29; N, 10.28. The residue (0.2 g.) from fraction-II was spotted in a line on fifty silicagel plates (20×20 cm., thickness 0.25 mm.) and developed with CCl_4-benzene (1:1 v/v), giving two colored bands. The lower band was scraped up from the plates and extracted with $CHCl_3$. The residue was recrystallized from benzene-EtOH to give 0.11 g. of tiny red needles (D-II), m.p. 196~197°(decomp.). TLC*[5] Rf 0.64. *Anal.* Calcd. for $C_{33}H_{48}O_4N_4$: C, 70.18; H, 8.57; N, 9.92. Found: C, 70.31; H, 8.24; N, 9.75.

Isomerization of D-II to D-I——A solution of D-II (0.01 g.) in benzene (1 ml.) and AcOH (0.01 ml.) was refluxed for 5 hr. After every one hour it was examined by TLC*[5], and complete isomerization was achieved during 5 hr.

*[4] Silicagel, 0.25 mm.; solvent, chloroform.
*[5] Silicagel, 0.25 mm.; solvent, CCl_4-benzene (1:1 v/v).
8) L. F. Fieser: J. Am. Chem. Soc., **75**, 3500 (1953).

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