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Odor Differences of 2-Methyl-4-[(*R*)-2-endo-bornyl]-cyclohexanone Stereoisomers: Synthesis, Separation by High-Performance Liquid Chromatography, and Elucidation of Absolute Configuration

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Synthesis and separation of the four stereoisomers of 2-methyl-4-[(*R*)-2-endo-bornyl]cyclohexanone by preparative high-performance liquid chromatography is described. The structure of these compounds is confirmed by their mass, proton magnetic resonance, and circular dichroism spectra, and also by means of isomerization of the axial α-methyl group in the cyclohexanone ring to an equatorial position. Only (*R*)-2-methyl-(*R*)-4-[(*R*)-2-endo-bornyl]cyclohexanone is found to have a "urinous" odor.

INTRODUCTION

The odor of 2-methyl-4-[(*R*)-2-endo-bornyl]cyclohexanone **(I)** is identical to the "urinous" odor of 5α-androst-16-en-3-one (*1*), which is believed to be the "primary" odor (*2*), and the existence of a specific receptor to 5α-androst-16-en-3-one is discussed (*3–6*). Earlier, a mixture of **(I)** stereoisomers was synthesized (*1*), but the authors did not separate the steroisomers which differed in arrangement and axial and equatorial positions of the α-methyl substituent in the cyclohexanone ring, because of the possible epimerization via enols, so that it remained unclear which of the stereoisomers had the "urinous" odor.

Since the presence of two asymmetrical carbons in the cyclohexanone ring of **(I)** is responsible for the existence of the four diastereoisomers **(Ia, Ib, Ic, Id),** we expected them to be separated by means of high-performance liquid chromatography. The chief aims of the present study were isolating all four **(I)** isomers; finding conditions that would keep them homogeneous; structural assignment; and odor evaluation.

EXPERIMENTAL PROCEDURES

All reagents and solvents were of reaction grade. *d*-Camphor was from a natural source (optical purity 100%). The solvents for HPLC were distilled before use.

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0045-2068/88 $3.00

HPLC was performed at room temperature with a DuPont 830 preparative liquid chromatograph equipped with a DuPont refractive index detector and a Hewlett–Packard 3380A integrator. For preparative mode detection, the flow was divided at a ratio of about 10 : 1 and the lesser of the flow was directed to the RI detector. A DuPont Zorbax SIL analytical column (0.46 × 25 cm) was used for analytical HPLC. Preparative HPLC was performed with a Chromapack-packed preparative column (2.27 × 25 cm) containing Lichrosorb SI 60 (7 μm, test efficiency 11320 theoretical plates). Elution was effected isocratically using the hexane–ethyl acetate mixture (98 : 2) for analytical (pressure, 1000 psi; flow rate, 2 ml/min) and preparative (800, 22) HPLC.

Infrared spectra were obtained on a Beckman Acculab VI spectrophotometer in CCL_4 (10% solutions). Mass spectrometry was carried out with an MX 1303 spectrometer (70 eV). Proton nuclear magnetic resonance (PMR)[2] spectra were measured in CCl_4 using a Varian HA-100D instrument (100 MHz, TMS), with $Eu(fod)_3$ as the shift reagent. Circular dichroism spectra was obtained on a Jouan III dichromater at 20°C in hexane.

Isomerization of **Ic** and **Id** was carried out with 5% methanolic potassium hydroxide for 24 h at room temperature.

2-Methyl-4-(2-bornylenyl)anisole (***III***). A solution of 2-methyl-4-bromoanisole (0.1 mol) in THF (20 ml) was added to a mixture of Mg (0.125 mol) and THF (15 ml) at 50°C over 1 h. After the solution was stirred at this temperature for 2 h, a solution of *d*-camphor (0.108 mol) was added under reflux and the reaction mixture was kept overnight at 20°C. The usual workup produced the crude oil, which was then heated at 50°C *in vacuo* (oil pump) until the unreacted camphor was removed. The reaction products were dissolved in chloroform (1 liter), passed through the short column with silica gel (100 g, activity 1), concentrated, and purified by column chromatography using silica gel (50 g) and hexane as an eluant to give 2.88 g (11.2%) of **III** as a colorless oil: ir 1610 (arom), 1245, 1140, 1045 ($-OCH_3$) cm^{-1}; PMR (δ) 0.76 (3H, s), 0.86 (3H, s), 1.06 (3H, s), 1.1–2.0 (4H, m), 2.18 (3H, s), 2.31 (1H, t, J = 3 Hz), 3.82 (3H, s), 5.86 (1H, d, J = 3 Hz), 6.6–7.2 (3H, m) ppm; mass spectrum (%) 256 (M^+, 80), 241(48), 228(100), 213(62), 199(16).

2-Methyl-4-[(R)-2-endo-bornyl]anisole (***IV***). A solution of 2-methyl-4-(2-bornylenyl)anisole (**III**) (8.2 mmol) in dry ether (40 ml) was added portion-wise to a mixture of Na (34.7) mmol) and liquid ammonia (130 ml). After 15 min of stirring, the usual workup gave 1.99 g (94.5%) of **IV**, mp 86.0–7.5°C (mp 89.0–90.8°C (1): ir 1625 (arom), 1258, 1145, 1050 ($-OCH_3$) cm^{-1}; PMR (δ) 0.65 (3H, s), 0.86 (3H, s), 0.95 (3H, s), 1.2–2.1 (7H, m), 2.18 (3H, s), 2.96 (1H, q, J = 12, 6 Hz), 3.80 (3H, s), 6.6–7.1 (3H, m) ppm; mass spectrum (%) 258 (M^+, 40), 149(44), 148(100), 135(38), 133(22), 95(27).

2-Methyl-4-[(R)-2-endo-bornyl]phenole (***V***). A mixture of 2-methyl-4-[(*R*)-2-endo-bornyl]anisole (**IV**) (6.1 mmol) and pyridinium hydrochloride (17.3 mmol) was heated for 4 h at 250°C in a seal tube, diluted with water, and extracted with CH_2Cl_2. The combined extracts were concentrated and chromatographed on silica

[2] Abbreviations used: PMR, proton magnetic resonance; psr, paramagnetic shift reagent; THF, tetrahydrofuran.

gel (60 g) in CH_2Cl_2 to produce 1.45 g (97.6%) of **V**, mp 112.0–3.5°C: ir 3625 (–OH), 1640 (arom), 1125 (–OH) cm^{-1}; PMR (δ) 0.63 (3H, s), 0.85 (3H, s), 0.95 (3H, s), 1.1–2.0 (7H, m), 2.15 (3H, m), 2.96 (1H, q, J = 12, 6Hz), 4.75 (1H, s), 6.4–7.0 (3H, m); mass spectrum (%) 244 (M^+, 32), 135(34), 134(100), 133(16), 121(40), 95(38).

A mixture of 2-methyl-4-[(R)-2-endo-bornyl]cyclohexanone stereoisomers (***Ia–Id***). Hydrogenation of 2-methyl-4-[(*R*)-2-endo-bornyl]phenole (**V**) (2.58 mmol) in ethanol (13 ml) over 200 mg fresh Ni-Raney catalyst at 160°C and 120 atm H_2 over 5 h produced 0.522 g (80.8%) of a mixture of corresponding cyclohexanols which was treated without purification with 0.6 g of pyridinium chlorochromate in dry CH_2Cl_2 (25 ml) over 24 h at room temperature. The usual workup and flash chromatography on silica gel (5 g, hexane–chloroform, 1 : 1) gave 0.479 g (92%) of a mixture of **Ia–Id** as a colorless oil: ir 1730 (C=O) cm^{-1}.

Isolation of individual stereoisomers by preparative HPLC. A mixture of stereoisomers **Ia–Id** (50 mg) was injected into a preparative HPLC system and individual components were collected manually, concentrated, and rechromatographed. It took at least two cycles of rechromatography for the complete purification of each component, homogeneity being confirmed by analytical HPLC on a Zorbax SIL column.

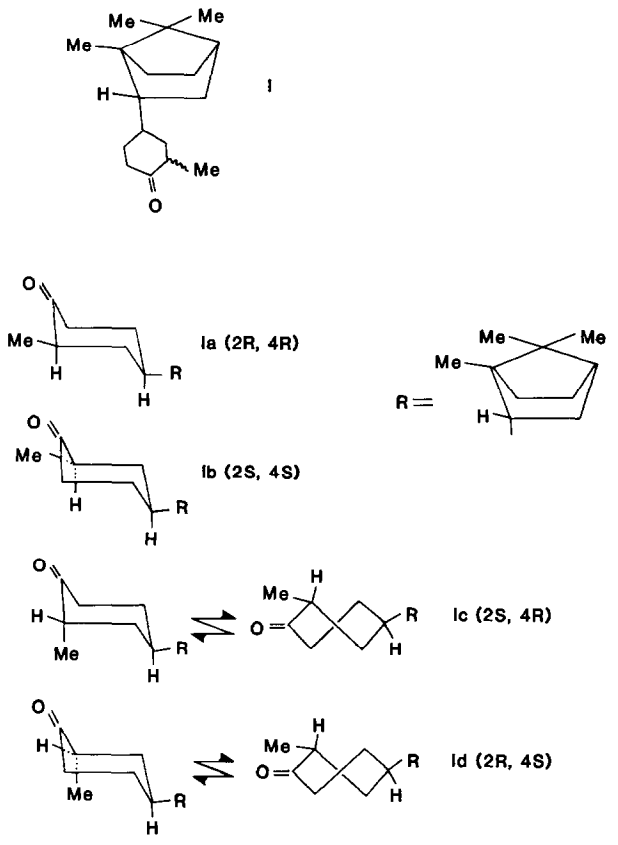
Odor evaluations. The quality of the odor and relative odor strength were determined by using dilute solutions of stereoisomers **Ia–Id** according to (*7*). The test panel consisted of 10 panelists capable of perceiving the odor of 5α-androst-16-en-3-one (*1*).

Six dilute solution of the compound under investigation were prepared in ethanol (1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} mg/ml) and 10 μl of each solution was applied to the coded cellulose filter strips, which were handed out to the panelists (a strip with pure ethanol served as a control). The panelists were asked to determine the minimal perceptible concentration having the odor of androstenone or some other odor. According to this procedure, the olfactory threshold concentration for androstenone was 10^{-4} mg/ml and its relative odor strength was assumed as 1000.

RESULTS AND DISCUSSION

We obtained 2-methyl-4-[(*R*)-2-bornyl]anisole (**IV**, Scheme I) according to Theimer's synthetic approach (*1*), modified at the tertiary alcohol **(II)** dehydration step. *d*-Camphor was treated in tetrahydrofuran with a Grignard magnesium reagent, obtained from 2-methyl-4-bromanisole. Alcohol **(II)** without purification was dissolved in chloroform and passed through a short column of silica gel; the resulting bornylenanisole **(III)** was purified by conventional column chromatography in hexane. The chemical reduction of **(III)** gave only an endo-camphor derivative **(IV)** which was heated with pyridinium hydrochloride to yield **V.** The endo-bornyl phenole **(V)** was hydrogenated over a Ni-Raney catalyst and oxidized with a Corey reagent (*8*) to the mixture of stereoisomers **Ia–Id.**

In using this synthetic route, we assumed that in the mixture of isomers **Ia–Id**



SCHEME I

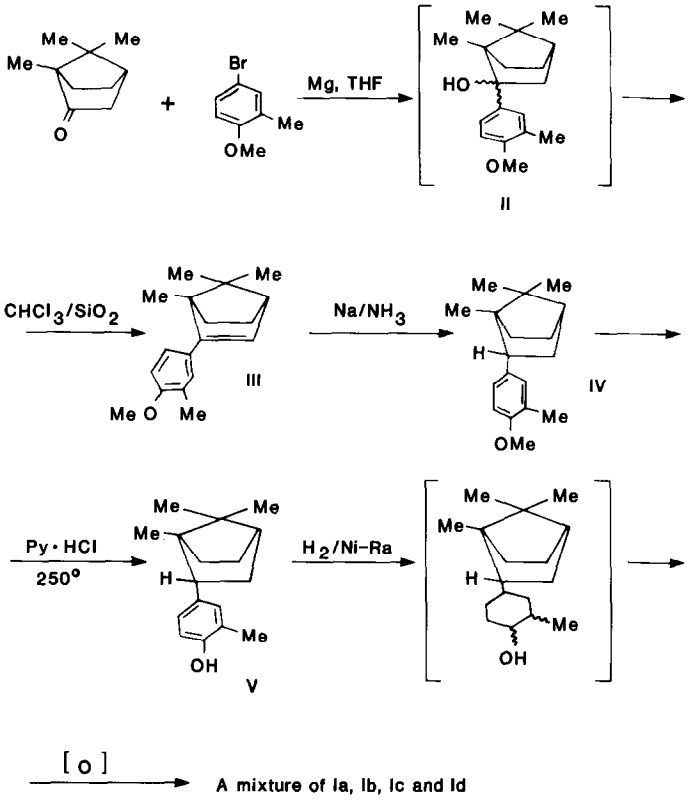
with an energetically more preferable equatorial bornyl substituent, the more stable **Ia** and **Ib,** which possess an equatorial α-methyl group, should predominate. In this case, **Ia** and **Ib** should have chair conformation of the ring, whereas **Ic** and **Id** can exist as mixtures of chair and twist-boat forms (*9*).

A mixture of ketones **Ia–Id** was homogeneous at different modes of chromatography, but HPLC on a Zorbax SIL analytical column revealed the presence of four peaks (Fig. 1), with the former two prevailing.

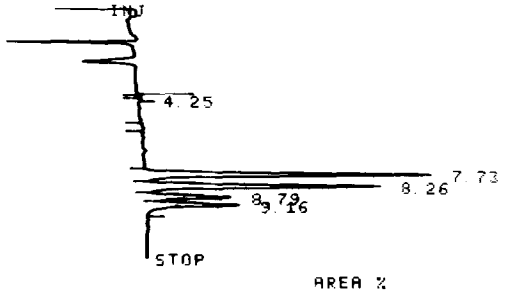
Compounds from peaks 1–4 (Fig. 1) were separated by repeated preparative HPLC on a Chrompack preparative column with Lichrosorb SI 60 in 2% ethyl acetate in hexane. At least two steps of rechromatography were needed to obtain each peak as an individual compound (purity ≥99%, RI detector, integrator).

Mass spectra of **Ia–Id** were quite similar with molecular ions at m/z 248 and relative intensities of the ions differing slightly (Fig. 2). The type of fragmentation corresponded to the structure **(I).** PMR spectra of **Ia–Id** were also practically identical (spectra A, Figs. 3–6), indicating that these compounds have only minor stereochemical differences.

To determine the absolute configuration of the α-methyl substituent in **Ia–Id,**



SCHEME I—*continued.*



RT	TYPE	AREA	AREA %
4.25		1464	200.3
7.73		283405	38.77
8.26	M	244344	33.43
8.79	M	92883	12.71
9.16	M	108829	14.89

HP 3380A
DLY 4. STOP OFF REJECT OFF
MV/M 3.00 ATTN 64

FIG. 1. Separation of stereoisomers of 2-methyl-4-[(*R*)-2-endobornyl]cyclohexanone **(Ia–Id)** by analytical HPLC (sample, 200 μg; attenuation, 64). Peaks having retention times of 7.73, 8.26, 8.79, and 9.16 min contain **Ia, Ib, Ic,** and **Id,** respectively.

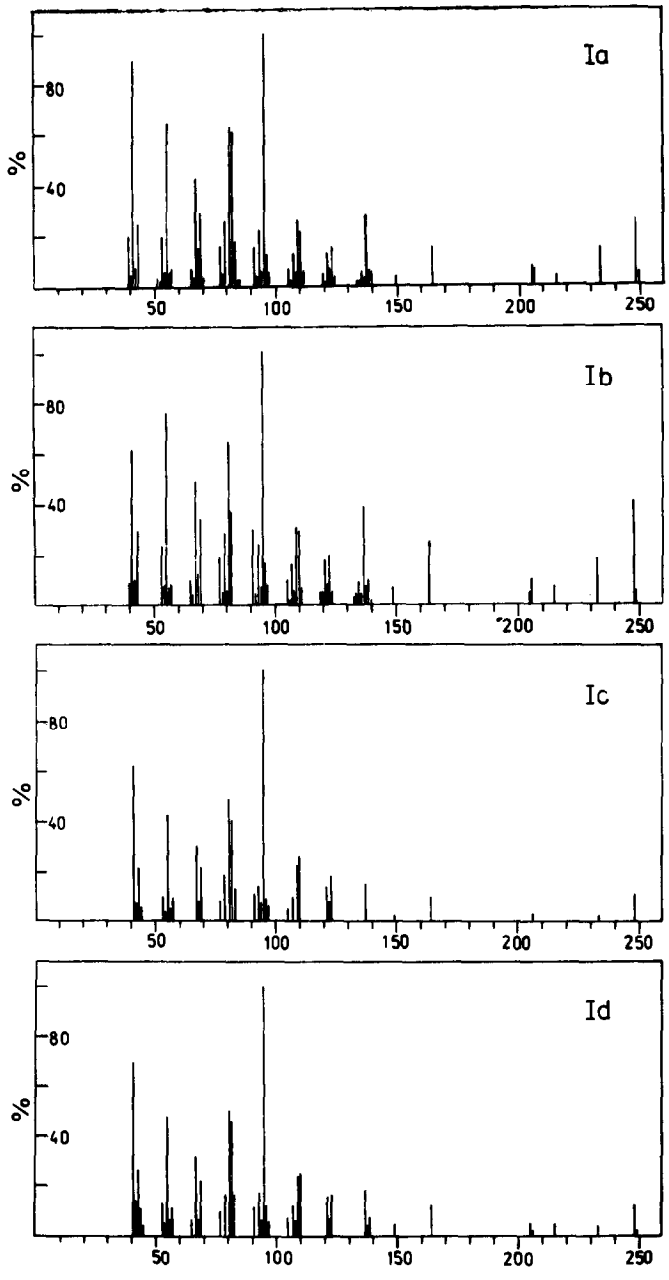


FIG. 2. Mass spectra of isomers **Ia–Id.**

circular dichroism data (presented in Fig. 7) were used. It was found that the CD spectra of the compounds from peaks 1 and 4 (Fig. 1) exhibited a negative, and from peaks 2 and 3 a positive Cotton effect. The amplitude and sign of the Cotton effects allows us, on the basis of the octant rule (*10*), to place α-methyl in an

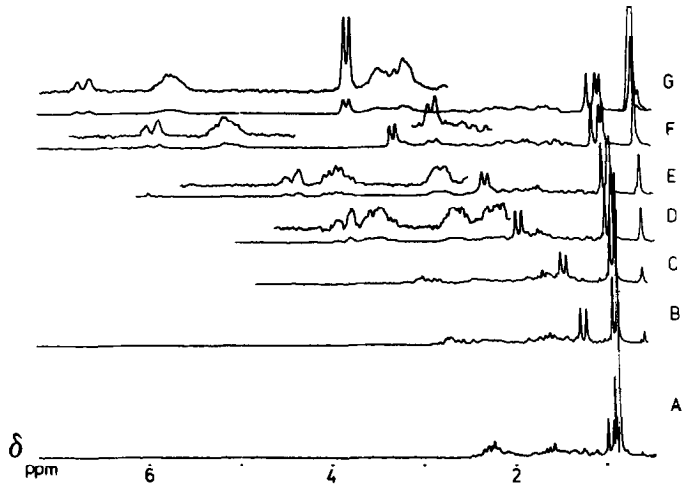


FIG. 3. The 100-MHz PMR spectrum of **Ia** (A) without a shift reagent; (B)–(G) with increasing concentration of $Eu(fod)_3$.

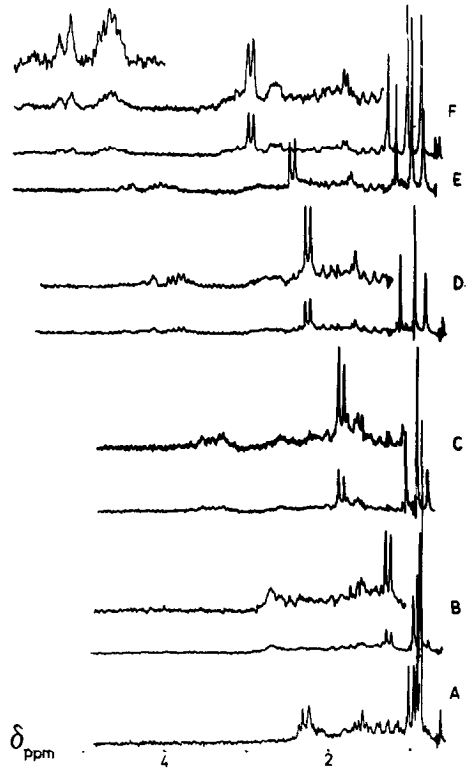


FIG. 4. The 100-MHz PMR spectrum of **Ib** (A) without a shift reagent; (B)–(F) with increasing concentrations of $Eu(fod)_3$.

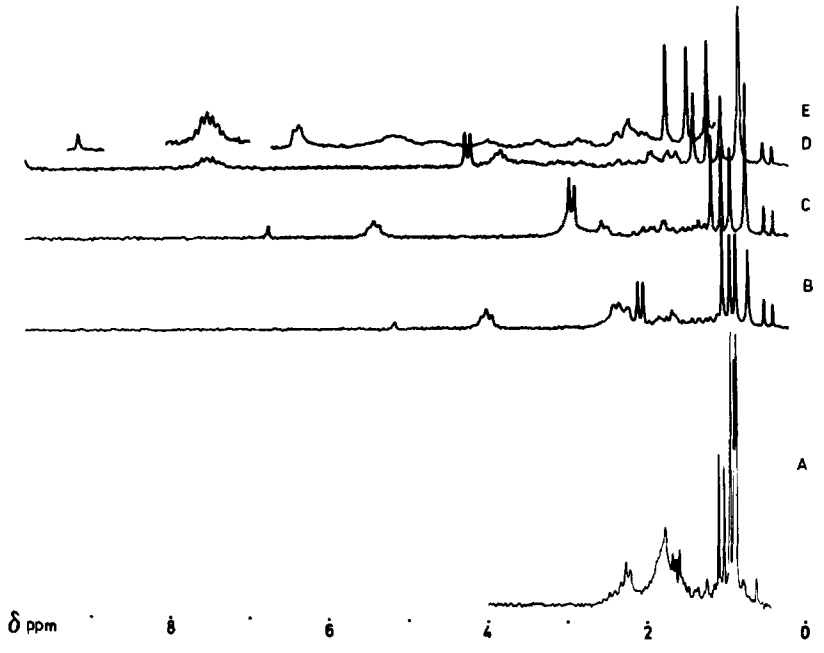


FIG. 5. The 100-MHz PMR spectrum of **Ic** (A) without a shift reagent; (B)–(E) with increasing concentrations of $Eu(fod)_3$.

equatorial (peaks 1 and 2) position and to assign them the **Ia** and **Ib** structures. The chemical transformation of a compound from peak 3 (Fig. 1) by dilute base treatment (5% methanolic KOH, 24 h, 20°C) produced a mixture of **Ia** (~90%) and a starting compound (~10%). The course of isomerization was controlled by analytical HPLC (retention time 7.73 and 8.79 min, Fig. 1). The same mixture of **Ib** and a compound from peak 4 (8.26 and 9.16 min, ~9 : 1) was obtained when the pure peak 4 was isomerized under these conditions. After base treatment, homogeneous **Ia** and **Ib** (peaks 1 and 2) showed no traces of isomerization. Axial α-methylcyclohexanones are known to epimerize under basic conditions (via enols), resulting in an equilibrium mixture of the equatorial and axial stereoisomers, with the former predominating. These facts, along with CD data, allowed us to assign the structures of **Ic** and **Id** to peaks 3 and 4, respectively.

Additional information concerning the stereochemistry of the α-methyl group and cyclohexanone ring conformation in **Ia–Id** was obtained from their PMR spectra in the presence of paramagnetic shift reagent (psr) $Eu(fod)_3$.

Two pairs of isomers give similar series of PMR spectra in the presence of psr, which are different, however, for each pair (Figs. 3 and 4 differ from Figs. 5 and 6). An analysis of the signals from α-methylene protons (δ = 3.8–4.2 ppm, Figs. 3F and 4D, broadened doublet and sestet, respectively) shows that the geminal coupling constant and one of two vicinal constants are equal to 14 Hz, whereas the second vicinal constant is equal to 6 Hz. The large value of the vicinal constant supports the trans-diaxial arrangement of the interacting protons ($\theta \approx 120°$),

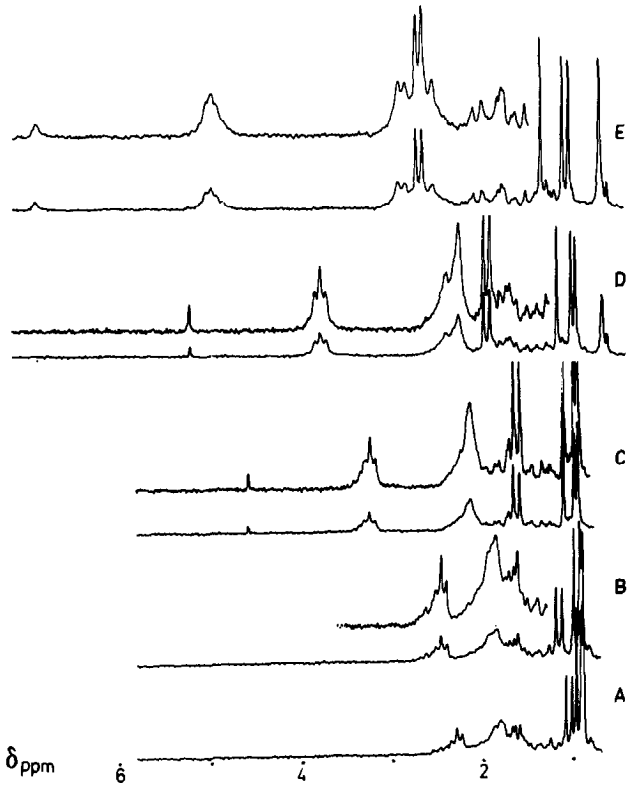


FIG. 6. The 100-MHz PMR spectrum of **Id** (A) without a shift reagent; (B)–(E) with increasing concentrations of $Eu(fod)_3$.

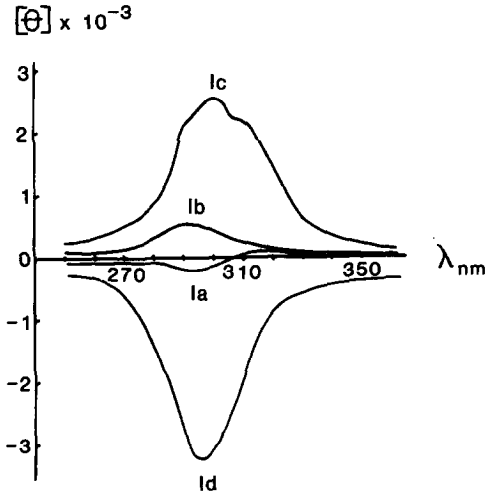


FIG. 7. CD spectra of isomers **Ia–Id.**

whereby it can be concluded that isomers **Ia** and **Ib** have chair conformation of the cyclohexanone ring. The shift rate ($\Delta\delta/\Delta C_{psr}$) for the equatorial proton signal is slightly higher than the shift rate for the axial signal, the latter coinciding with the shift rate of the signal from the proton, geminal to the α-methyl group. This signal behavior testifies to the equatorial position of α-methyl in **Ia** and **Ib.**

The shift rate of the signals from all α-protons in **Ic** and **Id** (Figs. 5 and 6) is equal. This fact makes it possible to assume that the distances between the axial and equatorial protons and the magnetic center are equal; e.g., the plane (where the carbonyl is arranged) divides the H–C–H angle in two. The resultant distortion of true chair conformation may be caused by the 1–3 interaction of the CH_3 group with two axial portions of the cyclohexanone ring. Therefore, we consider **Ic** and **Id** to exist at least as a mixture of chair and twist-boat conformers (~1 : 1), assuming that the bornyl group anchors the ring from the conversion to the other chair conformation. This effect may reflect the tendency of axial α-methyl to occupy a more stable, quasi-equatorial position.

Our conclusions regarding the structures of **Ia–Id** are in agreement with the general considerations on the stereochemistry of benzene ring catalytic hydrogenation in **VI;** in fact, more stable **Ia** and **Ib** are formed in greater amounts (38.8 and 33.4%, Fig. 1).

*Odor Evaluation of Compounds **Ia–Id***

It was found earlier (*1*) that an isomeric mixture of 2-methyl-4-[(*R*)-2-endo-bornyl]cyclohexanones has a specific "urinous" odor similar to that of 5α-androst-16-en-3-one and that its odor perception threshold is 1000 times greater than that of a steroidal ketone. Our panel experiments showed that only (*R*)-2-methyl-(*R*)-4-[(*R*)-2-endo-bornyl]cyclohexanone **(Ia)** had an "androsterone-like" ("urinous") odor. On the average, the relative odor strength for this compound was 1 (as compared with the value of 1000 for androsterone). Isomers **Ib–Id** (with 2*S*, 4*S*-, 2*S*, 4*R*-, and 2*R*,4*S*-configurations, respectively) had no "urinous," but only a "camphoraceous" odor. Dilute base isomerization only changed the "camphoraceous" odor of **Ic** to "urinous," while **Id** was unchanged. The fact that only one of the diastereoisomers of 2-methyl-4-[(*R*)-2-endo-bornyl cyclohexanone has a "urinous" odor of 5α-androst-16-en-3-one is in agreement with considerations regarding the chirality of a potentially existing receptor to this "primary" odor (*1*). The opinion that **Ia–Id** rapidly isomerize at room temperature (*1*) should be rejected, since in our experiments isomers **Ia–Id** were obtained as individual compounds and we failed to reveal any chromatographic, spectral, or organoleptic changes after keeping them at room temperature for several months.

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