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Note

Analytical and preparative separation of the *cis*- and *trans*-isomers of 4-(4'-*tert.*-butylcyclohexyl)-4-methylpentan-2-one by reversed-phase high-performance liquid chromatography

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Our studies of the relationships between chemical structure and odour has yielded individual *cis*- and *trans*-isomers of 4-(4'-*tert.*-butylcyclohexyl)-4-methylpentan-2-one (I and II). Synthesis of this compound was first described by Beets and Van Essen[1]. Later, Theimer *et al.*[2] reported that only the liquid *cis*-isomer I had a specific "urinous" odour, similar to the odour of 5α-androst-16-en-3-one[3], and that crystalline *trans*-isomer II was odourless. The authors did not specify how these substances had been obtained. They mentioned that solutions of the *trans*-isomer had a weak "urinous" odour, presumably because of the presence of *cis*-I as an impurity.

Here we describe the synthesis of a mixture of I and II, the separation of the isomers by preparative reversed-phase high-performance liquid chromatography (HPLC), structural evidence, spectral data and odour evaluation.

EXPERIMENTAL

Apparatus

HPLC was performed with a DuPont 830 preparative HPLC system equipped with a Rheodyne 7120 injector (loops 50 µl and 2 ml), a UV-photometer (254 nm), a DuPont refractive index (RI) detector and a Hewlett-Packard 3380 integrator. Stainless-steel (25 cm × 4.6 mm) columns packed with DuPont Zorbax C_8 (Ser. No. L947; test efficiency 13,890 theoretical plates) and Zorbax ODS (F3823; 15,130) were used for the analytical experiments. Adsorption and reversed-phase HPLC were performed with DuPont preparative columns (25 cm × 22.7 mm) packed with LiChrosorb SI 60 (particle diameter, d_p, 7 µm; test efficiency 11,320 theoretical plates) and LiChrosorb RP-8 (d_p 10 µm; 7490).

Gas–liquid chromatography (GLC) was performed with a Pye Unicam 104 gas chromatograph equipped with a flame ionization detector (FID) on a glass column (2 m × 4 mm) packed with 3% SE-30 or 10% free fatty acid phase (FFAP) on Chromosorb W at 180°C.

Combined GLC–mass spectrometry (MS) was carried out using an LKB 2091 mass spectrometer (70 eV), equipped with a capillary column (SE-30, 30 m × 0.2 mm) programmed from 100°C (5 min) to 260°C at 5°C/min.

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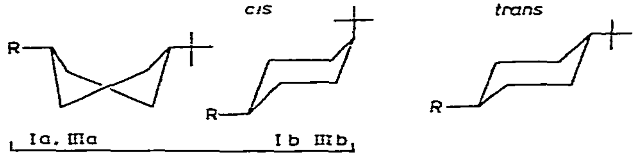
Infrared (IR) spectra (5% carbon tetrachloride solutions) were obtained using a Beckman Acculab VI spectrometer. ^{13}C Nuclear magnetic resonance (NMR) spectra were measured on a 20-MHz CFT-20 Varian spectrometer with tetramethylsilane internal standard.

Chromatographic conditions

The mobile phases were: adsorption preparative HPLC, hexane–chloroform (80:20), flow-rate 13 ml/min, pressure 840 p.s.i.; reversed-phase HPLC, water–acetonitrile (1:3), analytical columns, flow-rate 2 ml/min, pressure 1300 p.s.i., preparative column, flow-rate 19 ml/min, pressure 800 p.s.i. Fractions obtained in preparative reversed-phase HPLC were evaporated until a colourless emulsion was formed, then NaCl and hexane were added. The organic layer was separated and concentrated *in vacuo*.

RESULTS AND DISCUSSION

In previous papers[2,3] the synthesis of a mixture of *cis*- and *trans*-isomers of 4-(4′-*tert.*-butylcyclohexyl)-4-methylpentan-2-one (I and II) and their odour characteristics were described. The authors did not indicate how the individual isomers had been obtained. There was no spectral evidence in favour of the existence of any definite conformations for I and II.



Ia, IIIa Ib IIIb

I R = $C(CH_3)_2CH_2COCH_3$ II R = $C(CH_3)_2CH_2COCH_3$

III R = $C(CH_3)_3$ IV R = $C(CH_3)_3$

We obtained a mixture of I and II by successive benzene alkylation with mesityl oxide and *tert.*-butanol according to ref. 1, hydrogenation of the resulting 4-(4′-*tert.*-butylphenyl)-4-methylpentan-2-one over Raney nickel at 180°C and 160 atm H_2, oxidation by Corey reagent[4] and preparative HPLC of the reaction mixture (LiChrosorb SI 60).

The fraction isolated was not only homogeneous to adsorption HPLC but also to packed and capillary GC, and its IR, mass and ^{1}H NMR spectra corresponded to 4-(4′-*tert.*-butylcyclohexyl)-4-methylpentan-2-one. Only reversed-phase HPLC revealed the presence of the two peaks in this sample in a ratio of about 85:15, which could be separated on Zorbax ODS and C_8 analytical columns with α values of 1.27 and 1.065 respectively (Fig. 1a, b).

The separation strategy for preparative HPLC was selected according to Snyder and Kirkland[5]. During the first run (Fig. 2a) a homogeneous fraction A (oil) was isolated and after rechromatography of fraction B (Fig. 2b) we obtained peak I (the same substance as in fraction A) and peak II (colourless crystals, m.p. 46.8–47.8°C). Analytical HPLC confirmed that the compounds from peaks I and II were homogeneous (purity >99.9%, RI detector, integrator).

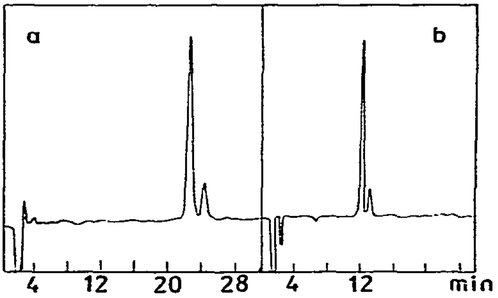


Fig. 1. Separation of *cis*- and *trans*-isomers of 4-(4′-*tert*.-butylcyclohexyl)-4-methylpentan-2-one by reversed-phase HPLC. Columns: 25 × 0.46 cm. Mobile phase: water–acetonitrile (1:3); flow-rate 2 ml/min. Pressure: 1300 p.s.i. Detector: RI, $2 \cdot 10^{-4}$ RI.u.f.s. a, Zorbax ODS; b, Zorbax C_8. Peaks: first = *cis*; second = *trans*.

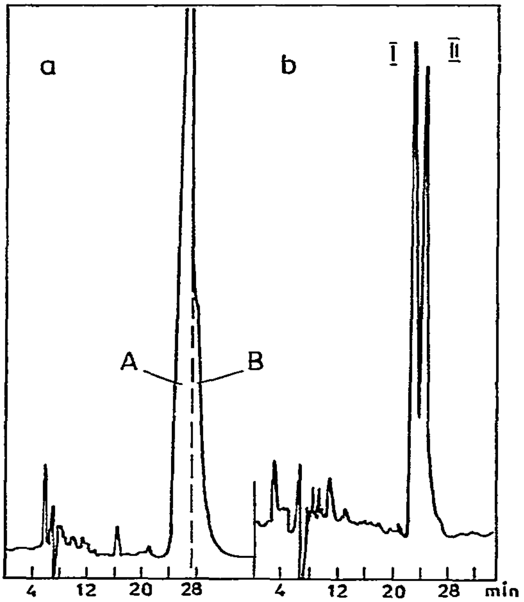


Fig. 2. a, Preparative HPLC separation of a mixture of I and II. Column: 25 × 2.24 cm, LiChrosorb 10 RP-8. Mobile phase: water–acetonitrile (1:3); flow-rate 19 ml/min. Pressure: 800 p.s.i. Detector: UV, 254 nm, 0.04 a.u.f.s. Sample: 493 mg. Fractions: A, 370 mg; B, 58 mg. b, Rechromatography of fraction B. Same conditions, except detector 0.01 a.u.f.s. Peaks: I = *cis*, 30 mg; II = *trans*, 25.4 mg; balance 94%.

Capillary GLC–MS showed that the retention time of these substances was identical but that their mass spectra differed slightly (Figs. 3 and 4). Based on these observations we presumed that the compounds from peaks I and II (Fig. 2b) were *cis*- and *trans*-isomers of 4-(4′-*tert*.-butylcyclohexyl)-4-methylpentan-2-one. These results are in agreement with the findings of Van Bekkum *et al.*[6], to the effect that catalytic hydrogenation of *p*-di-*tert*.-butylbenzene at 190°C leads to a mixture of *cis*- and *trans*-isomers of 1,4-di-*tert*.-butylcyclohexane (III and IV) in a ratio of about 8:2. The two sets of isomers have similar differences in melting points (20° and 94–96°C for III and IV and −5° and 46.8–47.8°C for I and II) and in IR spectra (see Fig. 5).

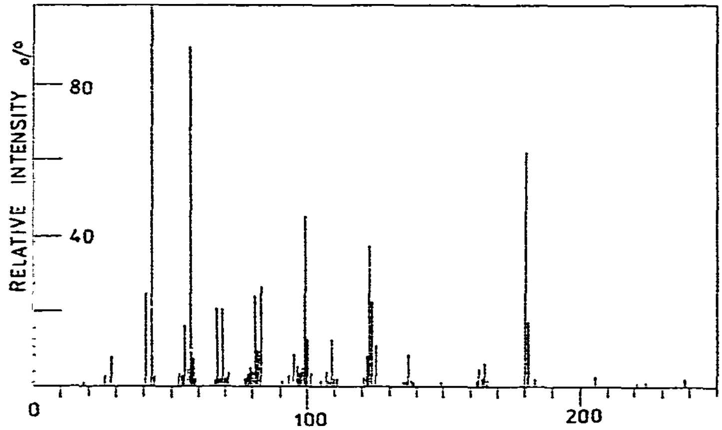


Fig. 3. Mass spectrum of isomer I: *m/e* (relative intensity in %) = 238 (0.6, M^-), 180 (62), 124 (22), 123 (38), 99 (45), 83 (28), 81 (24), 67 (20), 57 (90) and 43 (100).

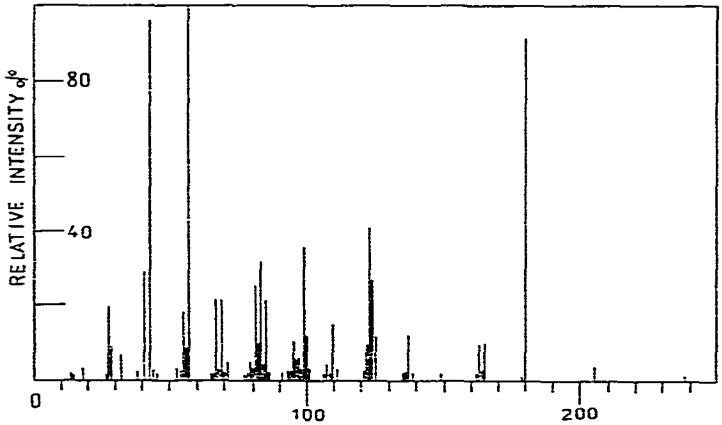


Fig. 4. Mass spectrum of isomer II: *m/e* (relative intensity in %) = 238 (1.1, M^+), 180 (90), 124 (27), 123 (41), 99 (36), 83 (32), 81 (27), 69 (23), 57 (100), 43 (96) and 41 (30).

Theoretically, the diastereomers I and II (as III and IV) may exist in various stereoisomeric forms, which differ in the conformations of the cyclohexane ring and in the axial and equatorial positions of the substituents. It was found earlier that stereoisomers with the bulky *tert.*-butyl group in the axial position were less stable[7]. From a X-ray structure analysis and a number of other methods, Van Bekkum *et al.*[6] showed that the *cis*- and *trans*-isomers of 1,4-di-*tert.*-butylcyclohexane existed in forms having equatorial substituents (IIIa and IV). However, it was found later that *cis*-isomer III should be considered as a mixture of the conformers IIIa and IIIb, with the former predominating (≈60%)[8–12].

In the ^{13}C NMR spectrum of *cis*-isomer I the signals of atoms C_5 and C_8 have lower chemical shifts compared to *trans*-isomer II (Table I). This is in agreement with Looms and Robinson's data[13] on the chemical shifts of atom $C_{1(4)}$ in the spectra of III and IV (δ_{TMS} = 42.8 and 48.4 ppm respectively), supporting their conclusion that

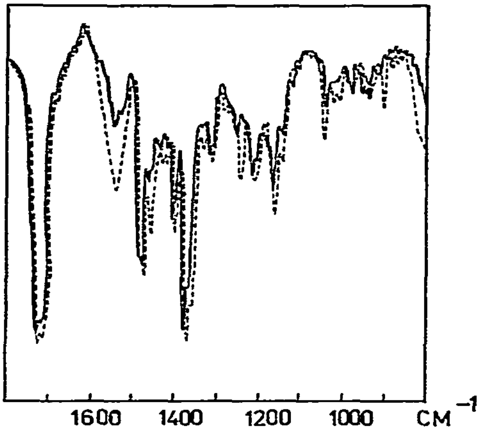


Fig. 5. Infrared spectra of *cis*- and *trans*-isomers I (———) and II (— — —) (5% carbon tetrachloride solutions).

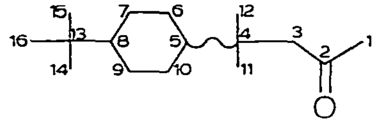
"... carbon atoms on twist rings are on average considerably more shielded to those in diastereoisomers in chair conformers with equatorial substituents". Similar results were obtained by Roberts *et al.*[14].

We conclude that the *trans*-isomer of 4-(4′-*tert.*-butylcyclohexyl)-4-methylpentan-2-one exists in form II, whereas its *cis*-isomer is a conformational mixture of forms Ia and Ib with the twist-form Ia predominating.

TABLE I

^{13}C NMR SPECTRAL DATA OF *cis*- AND *trans*-ISOMERS OF 4(4′-*tert.*-BUTYLCYCLOHEXYL)-4-METHYLPENTAN-2-ONE (I AND II)

s = Singlet; d = doublet; t = triplet; q = quartet; m = multiplet.



cis-I			*trans-II*		
δ_{TMS} (*ppm*)	*Multiplicity, "off-resonance"*	*No. of C atoms*	δ_{TMS} (*ppm*)	*Multiplicity, "off-resonance"*	*No. of C atoms*
52.59	t	3	52.61	t	3
42.07	d	5, 8	48.24	d	5, 8
41.31	d		46.67	d	
36.41	s	4, 13	35.87	s	4, 13
33.00	s		32.70	q	1
32.75	q	1	32.37	s	4, 13
27.68	m	11, 12,	27.58	m	11, 12, 14–16
24.76	m	14–16	24.94	m	6, 7, 9, 10
24.44	t	6, 7, 9, 10	209.50	s	2
23.06	t				
218.46	s	2			

In the odour evaluation experiments (ten panelists, sensitive to the odour of 5α-androst-16-en-3-one) we found that *cis*- and *trans*-isomers I and II both had "urinous" odour, but the odour perception thresholds were about 10^3 and 10^5-fold greater respectively than that for 5α-androst-16-en-3-one. Also, *trans*-isomer II, twice purified by preparative reversed-phase HPLC to a constant odour perception threshold, had a "urinous" odour, although its threshold was about 10^2-fold greater than that of *cis*-isomer I.

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