Conformational equilibria in amino steroids derived from fusidic acid

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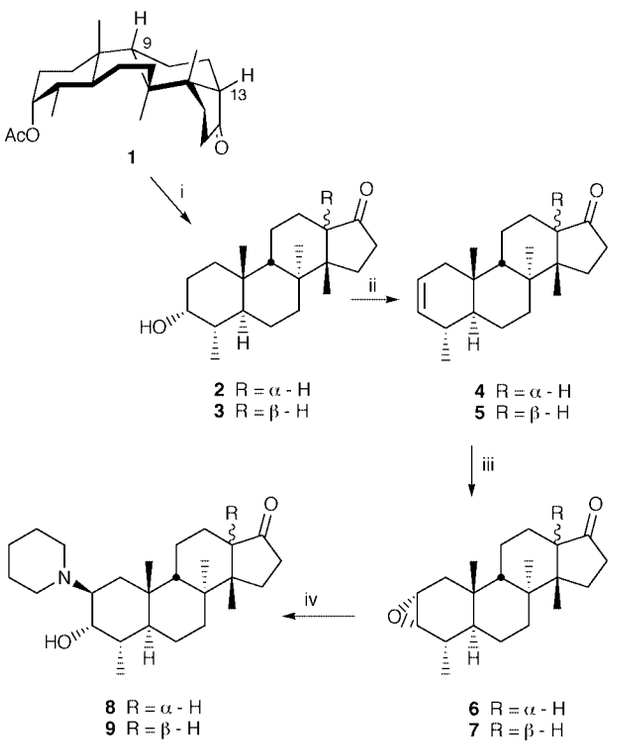
The conformation of ring A of the 3α-hydroxy-2β-piperidino steroid **9**, derived from fusidic acid, has been investigated by ^{1}H NMR and is determined by the polarity of the medium: it is primarily in a twist-boat conformation in a non-polar solvent and a chair conformation in a polar solvent.

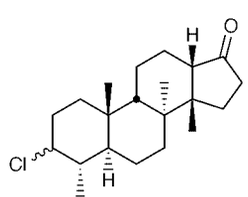
Fielding and co-workers found that the conformations of ring A of 3α-hydroxy-2β-morpholino-5α-androstan-17-one[1] and a number of related common steroids[2] are dependent on the polarity of the solvent. They determined that these steroids existed in a ring A twist-boat conformation in a non-polar solvent, whereas in a polar solvent they existed primarily in a chair conformation. This behavior has important implications for drug design and drug transport and is applicable to biologically active molecules such as the neuromuscular blocking agent, ORG 9426.[3] Although this more recently developed neuromuscular blocking agent is not as potent as vecuronium halides,[4] it shows more rapid onset of action leading to the postulate that the morpholino substituent confers novel conformational properties on the steroid which improve drug transport properties. For this reason, we chose to investigate analogues derived from the unusual 9β-steroid series in which ring B is constrained in a boat conformation and in addition, the 19-methyl group has the potential to exert an uncommon steric effect on ring A *via* buttressing by the 9β-hydrogen.

We report here the synthesis of **9** and the effect of solvent polarity on the conformational equilibrium of ring A. The tetracycle **1** had been prepared previously from fusidic acid[5] by us[6] and was employed as starting material. Hydrolysis with potassium carbonate in hot methanol[7] occurred quantitatively but resulted in the formation of a mixture of the 13α- and 13β-epimers, **2**[7] and **3**[7] (Scheme 1). These were separable by flash chromatography. Subsequently, for reasons of economy, all reactions were undertaken on mixtures of 13α- and 13β-epimers. Dehydration was effected with thionyl chloride in boiling benzene[8] and led exclusively to a mixture of the 13α- and 13β-epimeric Δ^2-steroids **4** and **5**, respectively. The two proton multiplet at δ_H 5.50 for the H-2 and H-3 vinylic protons together with the appearance of the doublet due to the 4α-CH_3 at low frequency, δ_H 0.96, in the ^{1}H NMR spectra of both the C-13 epimers **4** and **5**, are consistent with the assignment as Δ^2-steroids. When this step was attempted with $POCl_3$ in pyridine at room temperature, a mixture was obtained which consisted of the 3β-chloro product, as a mixture of 3α- and 3β-epimers **10** and **11**, with lesser quantities of the dehydration products **4** and **5**.

Epoxidation of the mixture of **4** and **5**, with *m*-chloroperoxybenzoic acid occurred exclusively on the less hindered[6,9] α-face, with the formation of **6** and **7**. This assignment was made both on the basis of the ^{1}H NMR coupling constant, $J_{3\beta,4\beta} = 5.8$ Hz, which is consistent with a *cis* relationship between H-3 and H-4, and on the close similarity of our results to those reported for the α-epoxidation of Δ^2-24,25-dihydrofusidic acid.[10] Reaction of the α-epoxides with piperidine was unexpectedly slow. However, it was finally achieved by extended heating of the reactants in ethylene glycol.[11] The method of Savage and co-workers[12] was not effective. As anticipated, regioselective *trans*-diaxial epoxide opening led to the formation of a 2β,3α-diastereomeric mixture of **8** and **9**. None of the 2α,3β-diastereomer was detected. The pure diastereomer **9** was separated from this mixture of C-13 diastereomers by



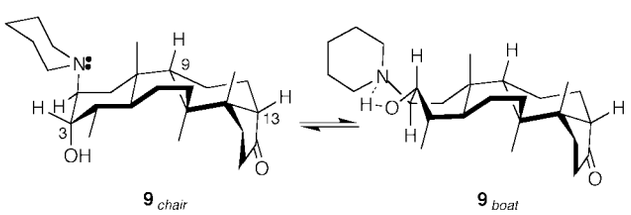
Scheme 1 *Reagents and conditions*: i, K_2CO_3, MeOH, Δ; ii, $POCl_3$, C_6H_6, Δ; iii, *m*-CPBA, Et_2O; iv, piperidine, $(CH_2OH)_2$, Δ.



wet flash chromatography. Our stereochemical assignments of the 13-proton in **8** and **9** were confirmed by applying the recently published method of Hanson and co-workers[13] to our spectral data.

The ^{1}H NMR of the 13β-epimer **9** was investigated in both deuteriochloroform and dimethyl sulfoxide-d_6. Both 1D and 2D ^{1}H NMR spectra at 270 MHz were used to assign the ^{1}H chemical shifts. The appearance of the signal corresponding to the 3β-proton was dependent on the solvent. Thus in deuteriochloroform the 3β-proton is seen as a doublet of doublets at δ_H 4.08, $J_{2\alpha,3\beta} = 11.3$ Hz and $J_{3\beta,4\beta} = 8.9$ Hz. The magnitude of these coupling constants are consistent with dihedral angles $\theta_{2\alpha,2\beta} \approx 180°$ and $\theta_{3\beta,4\beta} \approx 60°$, which correspond to a ring A twist-boat conformation. By comparison, in dimethyl sulfoxide-d_6, the 3β-proton is seen as a triplet at δ_H 3.79, $J_{2\alpha,3\beta} = J_{3\beta,4\beta} =$ 4.1 Hz. The magnitude of this coupling constant is consistent with dihedral angles $\theta_{2\alpha,3\beta} \approx \theta_{3\beta,4\beta} \approx 60°$ and corresponds to a ring A chair conformation.

These contrasting effects in chloroform and dimethyl sulfoxide are most probably due to a fine balance between intramolecular H-bonding, intermolecular H-bonding and ring strain. It has been calculated by Fielding and Grant[1] using MM2 calculations[14] that in the related 2,3-disubstituted androstane system there is only a 2.3 kcal mol^{-1} energy difference between the ring A twist-boat and ring A chair conformations. It is probable that, on the basis of the similarity of our results with those of this androstane system, similar energetics are involved. Thus, subtle effects are sufficient to shift the equilibrium in favour of either $\mathbf{9}_{chair}$ or $\mathbf{9}_{boat}$ (Scheme 2).



Scheme 2

This small energy difference is no doubt due to stabilisation of the twist-boat by intramolecular H-bonding and destabilisation of the chair conformation by the three axial substituents which nonetheless can now be stabilised by intermolecular H-bonding (Scheme 2).[15] In the non-polar chloroform, stabilisation *via* intramolecular H-bonding between the 2β-N and the 3α-OH substituents in **9** forces ring A into a twist-boat conformation. In dimethyl sulfoxide, this strained conformation relaxes into the ring A chair conformation and intermolecular H-bonding now becomes feasible. Its seems likely that ring A may be slightly flattened however, in order to alleviate some of the strain due to the three axial substituents in ring A.

It is of note that the presence of the strained ring B does not inhibit this ring A conformational equilibrium. The effect reported here appears to be as pronounced as in the 5α-androstane system,[1,2,15] which has the strain-free *trans-anti-trans* framework. Consistently, the 13α-epimer **8** also exhibits the same conformational equilibrium pattern as **9**. Unfortunately, investigation was limited to a mixture of **8** and **9** since we were unable to isolate **8** totally free of the epimer **9**. Nonetheless, it is clear that the behaviour of **8** in both $CDCl_3$ and DMSO-d_6 was virtually identical to that of **9**. Thus, the configuration of C-13 does not exert any major influence on the conformational equilibration of the A ring.

In conclusion, these results extend our knowledge of the dependence of the conformation of ring A of 2β-amino-3α-hydroxy steroids on solvent polarity. Since conformational changes in the steroid may facilitate the passage of the molecule through membranes or result in exposure of polar sites which may be selectively attracted to polar receptor sites,[1] these results provide additional implications for drug design. It is now apparent that the dramatic response of 2β-amino-3α-hydroxy steroids to solvent polarity is very tolerant of steric effects elsewhere in the molecule. This opens the possibility that neuromuscular blocking agents with potent biological activity analogous to ORG 9426,[3] vecuronium bromide[4] and pancuronium bromide[12,16] could be derived from fusidic acid and related or strained steroids with unusual topography.

Experimental

In general, the experimental procedures were the same as those we have described recently.[6]

The configurations of the 9β-androstanones **2** and **4**–**9** were assigned as 13α or 13β on the basis of the ^{13}C NMR chemical shifts of C-13 and C-17 of these compounds. We had noted that the ^{13}C NMR signals due to C-13 and C-17 in the known 13β-epimers were observed at higher frequency than those of the α-epimers. On this basis, the androstanones of indeterminate configuration were assigned as 13α or 13β. Androstanones **1**,[17] **2**[7] and **3**[7] had been synthesised previously but their ^{13}C NMR spectra had not been recorded and are therefore included here. The ^{13}C NMR spectrum of methyl fusidate had been assigned in full[18] and was also used for correlation purposes.

In addition, the ^{1}H NMR chemical shifts of the 13β-epimer **9** were assigned by means of 1D and 2D (H, H-COSY) spectra at 270 MHz in $CDCl_3$ and DMSO-d_6.

3α-Acetoxy-4α,8,14-trimethyl-18-nor-5α,8α,9β,13β,14β-androstan-17-one 1

Mp 143–145 °C (acetone–heptane) (lit.,[6,17] 143–145 °C) (Found: C, 76.4; H, 10.3. Calc. for $C_{23}H_{36}O_3$: C, 76.6; H, 10.1%); $[a]_D$ +18.2 (*c* 0.0146, chloroform, lit.,[6,17] +18); ν_{max}(KBr)/cm^{-1} 1715 and 1720; δ_H 4.90 (1H, m, 3-H), 2.04 (3H, s, OAc), 1.27 (3H, s, 32-CH_3), 0.94 (3H, s, 19-CH_3), 0.89 (3H, s, 18-CH_3) and 0.80 (3H, d, *J* 6, 30-CH_3); δ_C 219.92 (C-17), 170.89 (C), 74.19 (3-CH), 56.53 (13-CH), 47.04 (C), 45.97 (9-CH), 40.04 (C), 38.08 (4-CH), 36.82 (CH_2), 36.14 (C), 34.89 (5-CH), 30.85 (CH_2), 30.12 (CH_2), 29.87 (CH_2), 27.09 (CH_3), 26.94 (CH_2), 22.82 (CH_3), 22.19 (CH_3), 21.29 (CH_3), 20.88 (CH_2), 20.34 (CH_2), 19.84 (CH_2) and 15.42 (CH_2).

3α-Hydroxy-4α,8,14-trimethyl-18-nor-5α,8α,9β,13α,14β-androstan-17-one 2 and 3α-hydroxy-4α,8,14-trimethyl-18-nor-5α,8α,9β,13β,14β-androstan-17-one 3

The crude product was analysed by TLC using aluminium oxide as adsorbent and 20 : 1 dichloromethane–ethyl acetate as eluent. The two epimers (1 g) were separated by column chromatography using aluminium oxide (neutral, activity 4) (200 g) as adsorbent and 20 : 1 dichloromethane–ethyl acetate as eluent.

The first fraction was the less polar epimer **2**, ν_{max}(KBr)/cm^{-1} 3490, 1720 and 970; δ_H 3.78 (1H, m, 3β-H), 1.25 (3H, s, 32-CH_3), 0.94 (3H, d, *J* 6, 30-CH_3), 0.88 (3H, s, 19-CH_3) and 0.86 (3H, s, 18-CH_3); δ_C 218.59 (17-C), 71.69 (3-CH), 53.80 (13-CH), 47.08 (C), 45.26 (9-CH), 39.23 (C), 37.57 (4-CH), 36.48 (C), 34.75 (5-CH), 34.64 (CH_2), 31.86 (CH_2), 29.97 (CH_2), 28.55 (CH_2), 27.94 (CH_2), 22.25 (CH_2), 21.37 (CH_3), 20.83 (CH_2), 20.20 (CH_3), 19.86 (CH_2), 17.13 (CH_3) and 15.99 (CH_3).

The second fraction was the more polar epimer **3**, mp 153–155 °C (acetone–heptane) (lit.,[7] 159–161 °C) (Found: C, 79.3; H, 10.7. Calc. for $C_{21}H_{34}O_2$: C, 79.2, H, 10.8%); ν_{max}(KBr)/cm^{-1} 3600, 3400, 1710 and 970; δ_H 3.73 (1H, m, 3-H), 1.28 (3H, s, 32-CH_3), 0.94 (3H, s, 19-CH_3), 0.88 (3H, d, *J* 6, 30-CH_3) and 0.86 (3H, s, 18-CH_3); δ_C 220.05 (C-17), 71.41 (3-CH), 56.57 (13-CH), 47.06 (C), 45.91 (9-CH), 39.96 (C), 36.93 (4-CH),

36.78 (CH_2), 36.42 (C), 36.03 (5-CH), 31.19 (CH_2), 29.88 (CH_2), 29.85 (CH_2), 29.45 (CH_2), 26.94 (CH_3), 23.02 (CH_3), 21.98 (CH_3), 20.89 (CH_2), 20.34 (CH_2), 19.82 (CH_2) and 15.79 (CH_3).

4α,8,14-Trimethyl-18-nor-5α,8α,9β,13α,14β-androst-2-en-17-one 4 and 4α,8,14-trimethyl-18-nor-5α,8α,9β,3β,14β-androst-2-en-17-one 5

Freshly distilled thionyl chloride (14 ml, 209.3 mmol) was added to a solution of **2** and **3** (4.59 g, 14.4 mmol) in distilled benzene. The solution was heated under reflux 45 min. Work-up followed by flash chromatography (dichloromethane–heptane 3:1) afforded the products **4** and **5** (2.38 g, 53%).

The first fraction was the less polar 13β-epimer **5**, mp 160.5–163 °C (acetone–heptane) (Found: C, 83.6; H, 10.8. $C_{21}H_{32}O$ requires: C, 83.9; H, 10.7%); $[\alpha]_D$ +101.5 (*c* 0.0463); ν_{max}/cm^{-1} 1715 and 1670; δ_H 0.83 (3H, s, 18-H), 0.85 (3H, s, 19-H), 0.94 (3H, d, *J* 6, 30-H), 1.30 (3H, s, 32-H) and 5.50 (2H, m, 2-H and 3-H); δ_C 19.17 (CH_3), 19.33 (CH_2), 19.48 (CH_2), 20.93 (CH_2), 25.05 (CH_3), 26.52 (CH_3), 26.81 (CH_3), 30.31 (CH_2), 32.54 (5-CH), 34.56 (CH_2), 35.20 (C), 35.67 (CH_2), 36.71 (CH_2), 39.53 (C), 43.49 (4-CH), 46.19 (9-CH), 46.83 (C), 57.30 (13-CH), 125. 84 (CH), 134.41 (CH) and 219.85 (17-C).

The second fraction was the more polar 13α-epimer **4**, mp 95–97 °C (methanol–water) (Found: C, 84.0; H, 10.8. $C_{21}H_{32}O$ requires C, 83.9; H, 10.7%); ν_{max}/cm^{-1} 1715 and 1680; δ_H 0.83 (3H, s, 18-H), 0.88 (3H, s, 19-H), 0.98 (3H, d, *J* 6, 30-H), 1.18 (3H, s, 32-H) and 5.50 (2H, m, 2-H and 3-H); δ_C 17.07 (CH_3), 19.53 (CH_3), 20.18 (CH_2), 20.34 (CH_2), 20.74 (CH_3), 21.26 (CH_3), 21.76 (CH_2), 27.92 (CH_2), 32.33 (CH_2), 32.42 (5-CH), 34–61 (CH_2), 34.69 (C), 35.53 (CH_2), 39.03 (C), 43.34 (4-CH), 45.05 (9-CH), 46.95 (C), 54.32 (13-CH), 125.66 (CH), 134.63 (CH) and 218.54 (17-C).

2α,3α-Epoxy-4α,8,14-trimethyl-18-nor-5α,8α,9β,13α,14β-androstan-17-one 6 and 2α,3α-epoxy-4α,8,14-trimethyl-18-nor-5α,8α,9β,13β,14β-androstan-17-one 7

A solution of *m*-chloroperoxybenzoic acid (50–60%) (3.0312 g, 8.80 mmol) in dichloromethane was added dropwise to a stirred cooled solution of **4** and **5** (2.196 g, 7.32 mmol) in dichloromethane in an ice bath. The solution was stirred at room temperature 17 h. The solution was then washed with a sodium metabisulfite solution (10%), sodium hydroxide solution (10%), water and finally saturated sodium chloride solution. It was dried over anhydrous sodium sulfate. After removal of the solvent, the crude product was purified by flash chromatography (heptane–ethyl acetate, 5:1) to afford the purified products **6** and **7** (1.39 g, 60%).

The first fraction contained the less polar 13β-epimer **7**, mp 177–180 °C (acetone–hexane) (Found: C, 79.7; H, 10.3. $C_{21}H_{32}O_2$ requires C, 79.7, H, 10.2%); $[\alpha]_D$ +68.56 (*c* 0.018); ν_{max}/cm^{-1} 1720, 980, 945, 915, 835 and 800; δ_H 0.82 (6H, s, 18-H and 19-H), 1.09 (3H, d, *J* 6, 30-H), 1.28 (3H, s, 32-H), 3.03 (1H, m, 2-H) and 3.16 (1H, dd, *J* 3.8 and 5.6, 3-H); δ_C 15.99 (CH_3), 19.18 (CH_2), 19.36 (CH_2), 20.44 (CH_2), 22.64 (CH_3), 24.84 (CH_3), 26.59 (CH_3), 29.65 (CH_2), 30.29 (5-CH), 33.17 (CH_2), 34.77 (CH_2), 36.69 (CH_2), 39.16 (4-CH), 39.36 (C), 43.11 (9-CH), 46.70 (C), 53.71 (2-CH or 3-CH), 56.79 (13-CH), 58.17 (3-CH or 2-CH) and 219.42 (17-C).

The second fraction contained the more polar 13α-epimer **6**, mp 181–182 °C (diethyl ether–hexane) (Found: C, 79.9; H, 10.4. $C_{21}H_{32}O_2$ requires C, 79.7; H, 10.2%); $[\alpha]_D$ −82.37 (*c* 0.0113); ν_{max}/cm^{-1} 1710, 990, 835 and 800; δ_H 0.82 (3H, s, 18-H), 0.83 (3H, s, 19-H), 1.12 (3H, d, *J* 6, 30-H), 1.15 (3H, s, 32-H), 3.03 (1H, m, 2-H) and 3.16 (1H, dd, *J* 3.9 and 5.8, 3-H); δ_C 16.01 (CH_3), 17.07 (CH_3), 19.55 (CH_2), 20.39 (CH_2), 21.13 (CH_3), 21.62 (CH_2), 22.82 (CH_3), 27.99 (CH_2), 30.77 (5-CH), 32.29 (CH_2), 33.17 (CH_2), 34.45 (CH_2), 37.97 (4-CH), 38.89 (C), 42.88 (9-CH), 46.83 (C), 53.34 (13-CH), 53.71 (2-CH or 3-CH), 58.19 (3-CH or 2-CH) and 218.38 (17-C).

3α-Hydroxy-2β-piperidino-4α,8,14-trimethyl-18-nor-5α,8α,9β,13α,14β-androstan-17-one 8 and 3α-hydroxy-2β-piperidino-4α,8,14-trimethyl-18-nor-5α,8α,9β,13β,14β-androstan-17-one 9

A mixture of **6** and **7** (0.66 g, 2.089 mmol) was dissolved in a mixture of piperidine (6 ml) and ethylene glycol (24 ml). The solution was heated to reflux 24 h. Work-up followed by flash chromatography (ethyl acetate–methanol, 10:1) afforded a mixture of **8** and **9** (0.60 g) and purified 13β-epimer **9** (0.07 g) (total 0.67 g, 79%), mp 132–135 °C) (aq. acetone) (Found: C, 77.6; H, 10.9; N, 3.4. $C_{26}H_{43}NO_2$ requires C, 77.8; H, 10.8; N, 3.5%) (Found: M^+, 401.3295. $C_{26}H_{43}NO_2$ requires M, 401.3298); ν_{max}/cm^{-1} 3380 and 1725; $\delta_H(CDCl_3)$, 0.84 (3H, s, 18-H), 0.94 (3H, s, 19-H), 0.97 (3H, d, *J* 6, 30-H), 1.27 (3H, s, 32-H), 2.47 (2H, m, CH_2N), 2.72 (2H, m, CH_2N), 2.88 (1H, m, 2α-H) and 4.08 (1H, dd, *J* 8.9 and 11.3, 3-H); δ_H(DMSO-d_6) 0.78 (3H, s, 32-CH_3), 1.71 (1H, m, 4β-H), 2.23 (1H, m, 2α-H), 2.35 (2H, m, CH_2N), 24.4 (2H, m, CH_2N) and 3.79 (1H, t, *J* 4.1, 3-H); $\delta_C(CDCl_3)$ 12.696 (CH_3), 19.24 (CH_2), 19.37 (CH_2), 22.38 (CH_2), 24.23 (CH_2), 24.80 (CH_3), 25.83 (CH_2), 26.69 (CH_3), 27.15 (CH_2), 27.42 (CH_3), 30.32 (CH_2), 33.18 (5-CH), 35.03 (CH_2), 35.36 (C), 36.75 (CH_2), 39.69 (C), 43.52 (4-CH), 45.98 (9-CH), 46.71 (C), 49.11 (CH_2), 56.96 (13-C), 62.35 (3-CH), 65.25 (2-CH) and 219.24 (17-C); δ_C(DMSO-d_6) 15.04 (CH_3), 19.22 (CH_2), 19.86 (CH_2), 24.37 (CH_3), 25.42 (CH_2), 25.99 (CH_3), 26.08 (CH_2), 26.15 (CH_3), 28.98 (CH_2), 29.21 (CH_2), 30.63 (CH_2), 32.52 (CH), 35.52 (CH_2), 36.33 (C), 37.52 (CH), 67.90 (CH) and 218.10 (C).

3α-Chloro-4α,8,14-trimethyl-18-nor-5α,8α,9β,13β,14β-androstan-17-one 10 and 3β-chloro-4α,8,14-trimethyl-18-nor-5α,8α,9β,3β,14β-androstan-17-one 11

$POCl_3$ (0.8 ml, 8.7 mmol) was added to a solution of **3** (0.22 g, 0.69 mmol) in dry pyridine (25 ml). The solution was left stirring for 30 h at 20 °C. Work-up followed by flash chromatography (dichloromethane) afforded **10** (0.114 g, 50%); ν_{max}/cm^{-1} 1710, 980, 970 and 740; δ_H 0.84 (3H, s, 18-H), 0.98 (3H, s, 19-H), 1.06 (3H, d, *J* 6, 30-H), 1.29 (3H, s, 32-H), 3.44 (1H, td, *J* 11.4 and 5.7, 3-H), 5.20 (1H, m, 2- or 3-H) and 5.50 (1H, m, 3- or 2-H) (ratio of signal at δ 5.20 to that at δ 5.50 was 2:1); δ_C 17.09 (CH_3), 19.91 (CH_2), 20.13 (CH_2), 21.60 (CH_2), 22.97 (CH_3), 23.24 (CH_3), 26.67 (CH_3), 29.59 (CH_2), 31.93 (CH_2), 33.96 (CH_2), 35.03 (CH_2), 35.55 (C), 36.62 (CH_2), 39.69 (C), 40.42 (CH), 45.72 (CH), 45.81 (CH), 46.85 (C), 56.80 (CH), 68.47 (CH) and 219.68 (C).

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