Structural Elucidation of Ring *B* Aromatic Sterols of the Soil Amoeba *Acanthamoeba polyphaga*

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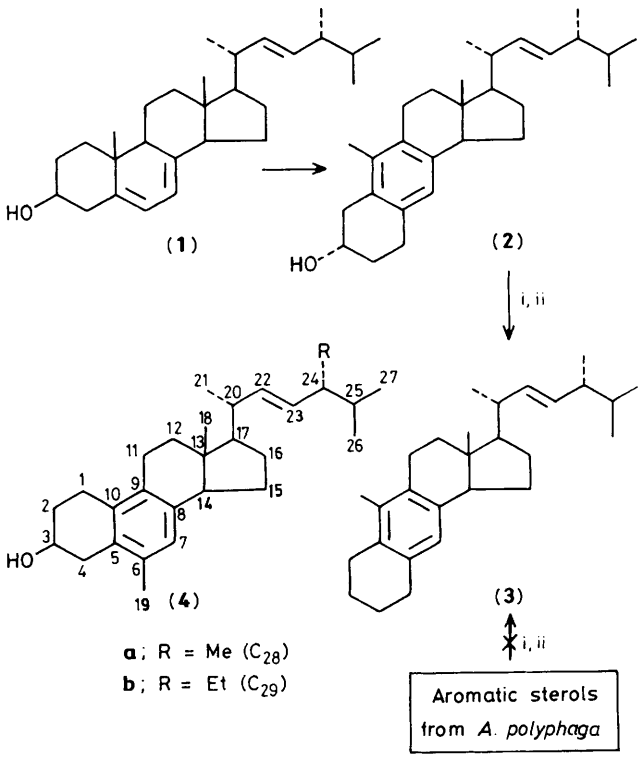
The structures of the two major aromatic sterols from the amoeba *Acanthamoeba polyphaga* have been determined by comparison with a synthetic anthrasteroid hydrocarbon and detailed ^{1}H and ^{13}C n.m.r. analyses as 19(10 → 6)-*abeo*-ergosta-5,7,9,22-tetraen-3β-ol and 19(10 → 6)-*abeo*-stigmasta-5,7,9,22-tetraen-3β-ol.

The widely distributed soil amoeba *Acanthamoeba polyphaga* has received recent attention after the discovery that several of its strains are pathogenic to vertebrates.[1,2] As in many protozoa and in spite of their importance as lipids, its sterols remained largely unstudied. Interestingly, as companions of more classical $\Delta^{5,7}$-sterols, we recently isolated from a cell-free system substantial amounts of C_{28} and C_{29} (depending on their side-chain) sterols with an aromatic *B* ring.[3] Concerning these compounds, as in a previous study on the closely related *Acanthamoeba castellanii*,[4] the usual spectroscopic analyses could not decide between a skeleton derived from a phenanthrene or from an anthracene, the later type being found for instance in some hydrocarbons of sediments.[5] We report here the full elucidation of their structure. Since the



Scheme 1. *Reagents:* i, Me_2SO, dicyclohexylcarbodiimide, pyridinium toluene-*p*-sulphonate, 40 °C, 24 h; ii, $N_2H_4 \cdot H_2O$, 120 °C, 90 min, then KOH, diethylene glycol, 220 °C, 4 h. The anthrasterol (**2**) was synthesised by the method in ref. 6.

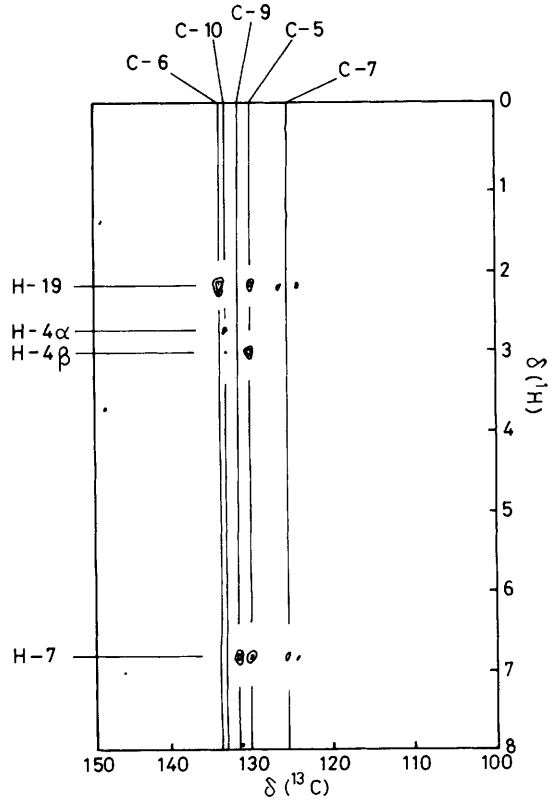


Figure 1. Part of the 2D correlated ^2J and ^3J heteronuclear ^1H and ^{13}C n.m.r. spectrum of aromatic steroids (**4**) from *Acanthamoeba polyphaga*.

differences between the two aromatic sterols (**4a**) and (**4b**) involved only a slight modification of the side chain easily recognized by n.m.r. spectroscopy, the structure of the tetracyclic moiety was determined on the natural mixture (130 mg) of (**4a**), 30%, and (**4b**), 70%, obtained using a cell-free system from a 60 l culture.

Scheme 1 shows the preparation of the anthrasteroid hydrocarbon (**3**) from ergosterol (**1**) which enabled us to rule out the anthrasteroid structure for the natural products. This preparation involves the already described rearrangement from a phenanthrasteroid to an anthrasteroid[6] followed by removal of the hydroxy group *via* its oxidation to a ketone by Moffat's method[7] and then Wolff–Kishner reduction. A similar reduction of the hydroxy group was carried out on the natural aromatic sterols but the C_{28} component led to a hydrocarbon different from (**3**) on g.c. analysis.

An anthrasteroid structure thus being out of the question, the location of the aromatic methyl group remained to be clarified and was determined by high-field n.m.r. spectroscopy. Assignments of proton signals† were made on the basis of the ^1H n.m.r. (400 MHz; $CDCl_3$) spectrum together with a 2D homonuclear correlated (COSY) experiment; carbon atom signals were attributed after comparison of the ^{13}C n.m.r. (100 MHz; $CDCl_3$) spectrum‡ with those of closely related compounds,[8] after careful examination of the non-decoupled spectrum and of the decoupled spectrum with and without spin echo (MULT) as well as the 2D correlated ^1J ^1H–^{13}C spectrum. The study of the non-decoupled spectrum led us to assign a value of 4 ± 1 Hz to the ^2J and ^3J coupling constants between ^1H and ^{13}C which was taken into account in the 2D ^2J and ^3J correlated ^1H–^{13}C spectrum. Part of this spectrum is shown in Figure 1 and indicates a coupling between ^{13}C-5 and ^1H-19 which favoured only structure (**4**) with the aromatic methyl group linked to C-6, the other possible position 7 giving a coupling constant between these two atoms ^4J nearly equal to zero.[9] This location at C-6 was confirmed by nuclear Overhauser effect (n.O.e.) experiments[10] in which irradiation of H-19 gave rise to a strong positive enhancement of the signal of H-4β, which is indeed spacially close to H-19 in the favoured half-chair conformation adopted by ring *A*,[6] as well as a slight positive enhancement of the 4α proton signal.

These aromatic sterols are still under investigation in our laboratory. They were isolated from a poorly studied group of eukaryotes: their distribution in other protozoa and their role are yet unknown. Their biosynthesis in addition (probably from the major $\Delta^{5,7,22}$-sterols of the ameoba) involves new and unprecedented enzymatic reactions.

† ^1H n.m.r. data (400 MHz; $CDCl_3$) for (**4**) (mixture): δ 6.745 (s, 1H, H-7), 5.215 (m, 1H, H-23 for C_{28} and C_{29}), 5.075 (dd, *J* 15.0 and 9.0 Hz, 1H, H-22), 4.120 (m, 1H, H-3α), 3.015 (dd, *J* 16.0 and 5.0 Hz, 1H, H-4β), 2.70 (m, 7H, H-1, H-11, H-14, H-20, H-24), 2.560 (dd, *J* 16.0 and 9.0 Hz, H-4α), 2.208 (s, 3H, H-19), 1.110 (d, *J* 6.5 Hz, 3H, H-21 for C_{29}), 1.095 (d, *J* 6.5 Hz, 3H, H-21 for C_{28}), 0.943 (d, *J* 7.0 Hz, 3H, H-28 for C_{28}), 0.872 (d, *J* 6.5 Hz, 3H, H-26 for C_{29}), 0.859 (d, *J* 6.5 Hz, 3H, H-26 for C_{28}), 0.847 (t, *J* 7.0 Hz, 3H, H-29), 0.845 (d, *J* 6.5 Hz, 3H, H-27 for C_{28}), 0.818 (d, *J* 6.5 Hz, 3H, H-27 for C_{29}), and 0.625 (s, 3H, H-18).

‡ ^{13}C n.m.r. data (100 MHz; $CDCl_3$) for (**4**) (mixture): C-1 (δ 24.1), C-2 (31.4), C-3 (67.5), C-4 (36.3), C-5 (129.9), C-6 (133.6), C-7 (125.3), C-8 (137.4), C-9 (131.3), C-10 (133.0), C-11 (24.9), C-12 (36.9), C-13 (41.5), C-14 (51.6), C-15 (29.4), C-16 (25.3), C-17 (54.9, 55.0), C-18 (11.2), C-19 (19.5), C-20 (40.8, 40.6), C-21 (21.0, 19.9), C-22 (138.0, 132.0), C-23 (129.6, 135.5), C-24 (51.2, 42.8), C-25 (31.8, 19.6), C-26 (18.9, 17.6), C-27 (21.2, 21.0), C-28 (25.1, 33.0), and C-29 (12.4,—). When two values are given, the first one is for the 24-ethyl derivative (**4b**), and the second for the 24-methyl derivative (**4a**).

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