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Novel Series of Diaromatic 14-Methyl Steroids Occurring in Petroleum

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Abstract: A novel series of unusual A/B diaromatic14-methylsteroids was identified by total synthesis of reference compound **1a**. They constitute the major aromatic hydrocarbon components from Pakistani biodegraded crude oil seeps. They probably derive from the aromatization of ring A functionalized 14-methyl steroid precursors present in green algae. Copyright © 1996 Elsevier Science Ltd

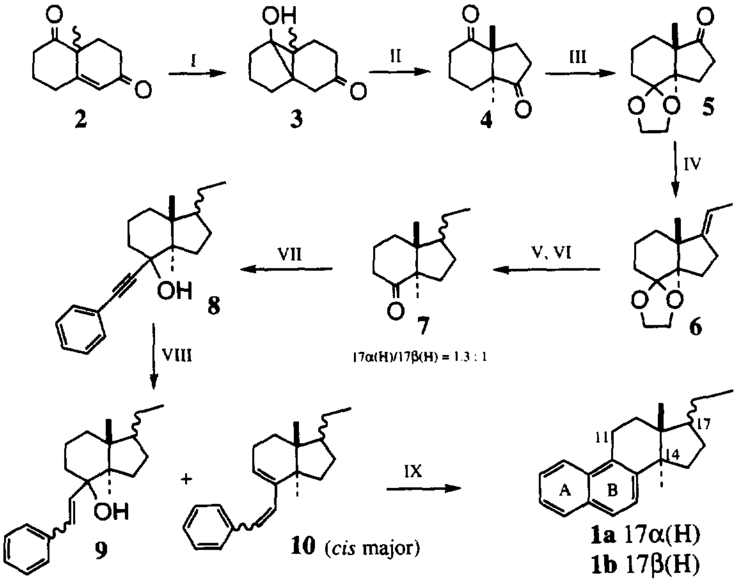
One major aspect of molecular organic geochemistry aims at elucidating the chemical structures of fossil organic matter present in soils, sediments and petroleum. In particular, the study of biological markers – molecules whose structures are sufficiently specific to relate them to their possible functionalized precursors in organisms – has proved a valuable tool to understand better the origins and the transformations of biological organic matter.[1] During the course of a study of the molecular changes occurring in a biodegraded seep oil from Pakistan exposed to weathering conditions, GC-MS analysis of the aromatic fractions revealed the presence of a major unknown series of diaromatic steroids (ranging from $C_{21}H_{26}$, **1a**, to $C_{31}H_{46}$, the C_{25} component being absent).

Close examination of the mass spectra of these new compounds[2] revealed the presence of a *m/z* : 193 fragment characteristic of an A/B diaromatic steroid hydrocarbon[3], a $(M-15)^+$ base peak indicating the loss of an easily cleaved methyl group, and similarities with the fragmentation pattern of ring B aromatic triterpenes, e.g. 25-norarbora-5,7,9-triene[4]. These indications led us to propose a 14α-methyl diaromatic steroid structure bearing a *C/D* ring *trans* junction, by analogy with lanosteroids.

In order to elucidate fully their structure, the total synthesis of the C_{21} homologue, 14-methyl-19-norpregna-1,3,5,7,9-pentaene **1a**, was undertaken. The construction of the hydrocarbon skeleton was achieved in a different way from the classical approaches for lanosterol[5] and 24,25-dihydrolanosterol.[6] Since no C(4)-demethylated natural product having a 14-methylsteroid-like structure was available, we chose Reusch's model for triterpene side-chain synthesis,[7a,b] which allowed us to build the *C/D* synthon **7** with the proper angular methyl substituents and side-chain before adding a phenylstyrene moiety and then aromatizing by photocyclization (scheme 1).

The *trans*-1,6-dimethylbicyclo(4.3.0)nonane-2,7-dione intermediate **4** was prepared in 2 steps from the racemic Wieland-Miescher ketone **2** by reductive cyclization,[8a] followed by a base-catalyzed rearrangement[8b] of the intermediate cyclopropanols, with a 36% overall yield. As methanolic rearrangements generally occur with high regioselectivity,[8c] only the *trans* isomer was detected. This was confirmed by homo- and heteronuclear NMR correlation studies (COSY, NOESY and ^1H-^{13}C correlations).

In a further stage, the carbonyl function of the 6-membered ring of dione **4** was selectively protected by ketalization[8d] and the side-chain introduced *via* a Wittig reaction,[5] which required the use of an excess of NaI to give the desired ethylene derivative **6** in 63% yield.



I. Li, NH_3:THF ; $(NH_4)_2CO_3$; II. KOH, MeOH ; III. 2-ethyl-2-methyl-1,3-dioxolane, ε TsOH ; IV. PPh_3CHCH_3Br, DMSO/NaH, NaI ; V. H_2 35 atm, PtO_2, AcOEt, 80°C, 12 hr. ; VI. Acetone, ε TsOH ; VII. Phenylacetylene, BuLi ; VIII. $LiAlH_4$; IX. hν, I_2, cyclohexane

Scheme 1.Synthesis of 14-methyl-19-norpregna-1,3,5,7,9-pentaene **1**.

Hydrogenation of the double-bond was tried using various classical methods but proved particularly sluggish, probably because of steric hindrance. Hydroboration-protonolysis[9] provided the reduced ketone **7** in poor yield. However, quantitative reduction of the double-bond was finally achieved by drastic hydrogenation in the presence of catalytic PtO_2 (35 atm. H_2, overnight, 80°C, AcOEt) leading to a mixture of two isomers at C(17) in a 1.3:1 ratio.

Synthon **7**, obtained after deprotection, was coupled with lithium phenylacetylide, followed by specific reduction with $LiAlH_4$[10](2.5 eq.) to yield a mixture of allylic alcohols **9** and dienes **10**, possibly resulting from the spontaneous *trans*-diaxial elimination of water from the isomer of **9** bearing an axial hydroxyl group. All our attempts to convert **9** to **10** failed : neither dehydration nor acetylation could be achieved. Indeed, hindered tertiary alcohols are, in some cases, reluctant to dehydrate.[11] Most attempts (Ac_2O/AcONa, Burgess dehydration reagent,[12] $POCl_3$-pyridine,[11] $CuSO_4$) led to rearranged dehydration products.

Finally, oxidative photocyclization[13] of dienes **10** was carried out as a dilute solution in cyclohexane in the presence of iodine (pyrex, high pressure mercury lamp, 2 h.) and afforded, after HPLC purification, the desired 14-methyl diaromatic steroids **1a** and **1b** as a mixture in a 3:1 ratio (20% yield) which were not separable by HPLC.[14] Isomers **1a** and **1b** can however easily be distinguished by GC (slight overlapping of the peaks on DB5). Hence, NMR structural studies were performed on the mixture of isomers : ^1H, ^{13}C, DEPT, ^1H-^1H PS-COSY, NOESY, HMQC and HMBC experiments led to the unambiguous structures as shown below (Table 1). The β-stereochemistry of the side-chain at position 17 was confirmed, in the major isomer **1a**, by nOe effects between H-17/CH_3-C(14) and H-17/H-12α . The long range W-type effect between H-18/H-12α in the COSY spectrum, along with the nOe effect between H-12β and H-18, indicate a preferential C-ring chair-like conformation.

Mass spectra of the synthetic steroid **1a** and of the geological compound were identical. Moreover, both compounds coeluted on three different capillary columns using high resolution GC (SUPELCOWAX 60m, DB5 60m and DB17 30m, J&W, i.d.=0.25mm, film thickness=0.1μm).

Table 1. ^{13}C- and ^{1}H- NMR chemical shifts for **1a** and **1b** (CD_2Cl_2, 500 MHz, δ in ppm relative to TMS).

C	**1a** δ(C)	**1a** δ(H)	**1a** δ(H)	**1b** δ(C)	**1b** δ(H)	**1b** δ(H)
1	123.64	7.95 J =8.6 Hz		123.55[a]	[b]	
2	125.98	7.46		124.39[a]	[b]	
4	128.80	7.78		126.28[a]	[b]	
5	132.61			[b]		
6	126.33	7.64 J =8.5 Hz		124.67[a]	7.63 J =8.3 Hz	
7	124.87	7.26		[a,b]	7.26	
8	144.97			145.9		
9	130.2			[b]		
10	132.2			[b]		
11	23.85	3.24 (α)	3.25	24.20	3.28 (α)	3.27
12	29.13	2.02 (α)	1.86	26.97	2.31 (α)	1.73
13	50.32			45.7		
14	44.67			49.7		
15	32.81	1.87 (α)	1.98	33.91	1.95 (α)	2.06
16	28.51	2.19 (α)	1.45	29.27	1.66 (α)	2.23
17	47.25	1.88		50.24	1.72	
18	16.76	0.60		27.31	0.73	
19	28.45	1.10		29.05	1.14	
20	24.44	1.53	1.21	30.14	1.32	1.34
21	13.79	0.97 J =7.4 Hz		13.90	0.92 J =7.3 Hz	

[a] Signals may be interchanged [b] Signals could not be clearly assigned

Although some sedimentary derivatives of lanosterol, euphol or cycloartenol have occasionally been observed in geological samples[15,16] – among them a *B* ring monoaromatic derivative of lanostane[16] – 4,4-demethylated 14α-methyl steroids (in particular the novel series of diaromatic steroids) have to our knowledge never been reported from sediments or oils. It is also noteworthy that the C-11 benzylic ketones obviously formed by the natural oxidation of the 14-methyl diaromatic steroid hydrocarbons are major constituents of the more polar "ketone" fraction of the oil seeps as shown by catalytic reduction of the latter (Pd/C; S. Lemoine, Ph.D. thesis, in preparation). Furthermore, the saturated counterparts were also detected in the "alkane" fraction and identified from their mass spectral data.[17] These results confirm that aromatization processes occur widely during sedimentation, many being microbially induced at an early stage of diagenesis.[18a] In this case, the methyl substituents at the *C/D* ring junction probably hinder further aromatization, as observed in the case of the *B* ring aromatized derivatives of isoarborinol and fernene.[4] Aromatization of ring *A* and *B* also indicates that a functionality located on the left hand side of the parent molecule initiated the process.

Since the diagenetic aromatization processes starting in the *A* ring of triterpenes are known to remove, in most cases, only one of the 4-methyl groups,[18a,b] the absence of a substituent on the aromatic *A* ring suggests that the precursor was itself 4,4-demethylated. Indeed, 4,4-demethyl-14α-methylated sterols have been observed in a wide variety of marine organisms,[19,20] land plants,[21-24] and marine, as well as lacustrine unicellular green algae e.g. *Chlorella emersonii*.[25]. Given the general importance of algal producers related to *Chlorella* to the fossil biomass[26] and the minor contribution of typical land plant biomarkers to our geochemical samples, it is likely that these new diaromatic 14α-methyl steroids derive from microscopic green algae. Furthermore, early stage biodegradation of the side chains, during deposition of the organic matter, is probably responsible for the predominance of the short chain compounds.

These apparently uncommon biomarkers are of geochemical interest since they provide a useful means for correlations of crude oils and could characterize specific types of biological deposits.

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