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14β,22*R*-Epithiosteranes, a Novel Series of Fossil Steroids Widespread in Sediments

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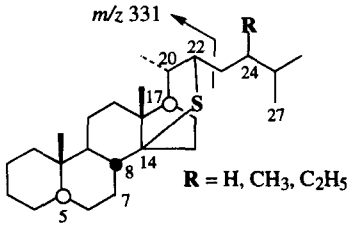
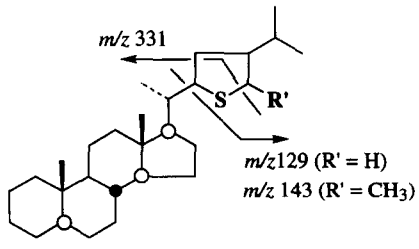
Abstract: C_{27}-C_{29} 14β,22*R*-epithiosteranes **1** were isolated from a Miocene sediment and their structures determined by 1D and 2D NMR. This novel series of sedimentary steroids presumably results from intramolecular incorporation of sulfur on steradiene precursors of algal origin.

Sulfur-rich organic matter usually occurs in natural anoxic environments where sulfate-reducing bacteria generate reduced inorganic sulfur species which can react with functionalized lipids from decaying organisms. This sulfur incorporation results predominantly in the formation of macromolecules cross-linked by sulfur bridges (intermolecular incorporation) and, to a minor extent, in the formation of free organo-sulfur compounds (intramolecular incorporation) of low molecular weight.[1] As the addition of sulfur takes place specifically at functionalized position(s), structural elucidation of organo-sulfur compounds is of major interest to obtain information on the sulfur incorporation mechanism(s), but also on the nature of the initial lipids which are often highly characteristic of their precursor organism(s) and may therefore be used as markers of source.[2]

Since the first identification of a sulfur-containing biological marker in sediments,[3] many organo-sulfur compounds having, for instance a linear, isoprenoid, hopane or sterane hydrocarbon skeleton have been characterized.[4] Among the latter, Schmid[5] reported the presence in a sulfur-rich crude oil of several series of thiophenes and thiolanes which have been identified by synthesis of standard molecules. An additional series of C_{28} and C_{29} steroid thiolanes, which was characterized in mass spectrometry by a base peak at *m/z* 129 (C_{28}) and *m/z* 143 (C_{29}), common fragments at *m/z* 317 and 331 and molecular ions at *m/z* 416 (C_{28}) and *m/z* 430 (C_{29}) has also been tentatively identified as tetrahydro-20-thienylpregnanes **2** and **3**.



1: C_{27} - C_{29} 14β,22*R*-Epithiosteranes

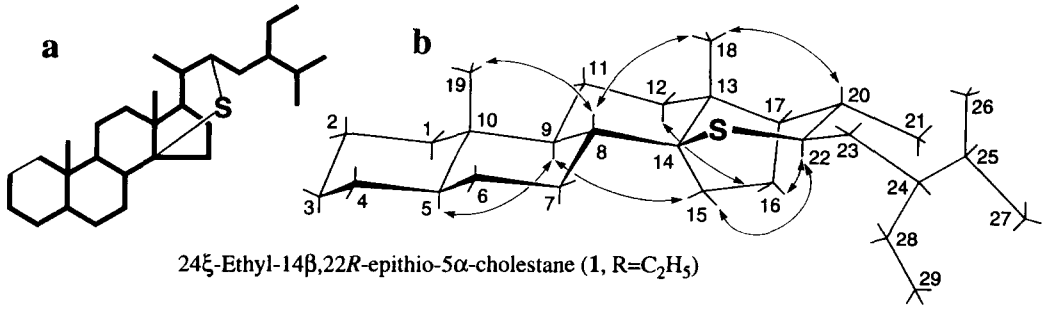


Tetrahydro-20-thienylpregnanes
[tentatively identified by Schmid (1986)]
2: R' = H (C_{28}); **3**: R' = CH_3 (C_{29})

The structures proposed were also in agreement with the fact that no C_{27} homologue of this series could be observed in the samples investigated. Since these tentative assignments, this novel series has been reported in

sediments of various origins such as the Miocene Monterey Formation (California, USA),[6] Timahdite bituminous shale (Morocco)[7] or the Messinian Gibellina sediments (Sicily, Italy).[8] In the latter samples, two C_{28} isomers (slightly separated by GC) and one C_{29} compound were also detected among the desulfurization products of S-rich macromolecules using Raney nickel,[8] indicating that the intramolecular sulfur atom must be located at a sterically hindered position. Moreover, two lower, C_{27} homologues of the same series were also present, which made the structure proposed by Schmid[5] unlikely. Therefore, identification of the C_{27}-C_{29} components has been carried out to reinvestigate their structure, determine their mode of formation and their possible origin(s).

The crude mixture (*ca.* 750mg) recovered upon Raney nickel desulfurization of 1g of organic extract from a Gibellina sample was fractionated by silica gel liquid chromatography (hexane as eluant), yielding *ca.* 33mg of a sulfide fraction. Further purification of this fraction by reversed phase HPLC (Du Pont Zorbax ODS; 9.4mm x 250mm, 7μm; acetone/methanol 60:40) led to the isolation of 1mg of the C_{28} steroids as a mixture of the two isomers and 2mg of the C_{29} compound. Similarly, 0.6mg of a mixture of the two C_{27} compounds could be obtained by combining the desulfurization products of several Gibellina samples. A first structural indication came from $Li/EtNH_2$ treatment[1d] of an aliquot of the C_{29} compound which yielded a mixture of 20*R*,5α(H),14α(H),17α(H)-24ξ-ethylcholestane and 20*R*,5α(H),14β(H),17α(H)-24ξ-ethylcholestane[9] in a 3:2 ratio, indicating that a C-S bond must be located at position 14 of the polycyclic hydrocarbon skeleton. Finally, the structure of the C_{29} compound was unambiguously established by 1D (^1H and ^{13}C) and 2D homonuclear (^1H-^1H: COSY and NOESY) and heteronuclear (^1H-^{13}C: HSQC and HMBC) NMR correlation experiments (Bruker ARX 500). The presence of six methyl, twelve methylene, eight methine groups and three quaternary carbon atoms, as well as the COSY, ^1H-^{13}C correlation experiments and the carbon connectivity deduced from the HMBC experiment established the steroidal skeleton structure (Fig.1a). In addition, the presence of three quaternary carbon atoms as well as the deshielded chemical shifts of the H-22 (2.7 ppm), H-15α (2.4 ppm) protons and of the C-14 (61.3 ppm) carbon atom indicated that C-S bonds were located at positions 14 and 22 (epithiosterane). The stereochemistry of the methyl groups and of the ring junctions could be determined by the nuclear Overhauser effects observed between 19-CH_3/H-8, H-9/H-5, 18-CH_3/H-8 whereas the nuclear Overhauser effects between 18-CH_3/H-20, H-22/H-15β, H-22/H-16β, H-15α/H-9 indicated that the sulfur-containing moiety had a 14β, 22*R*, 20*S* configuration (Fig.1b). Further confirmation comes from the high coupling constants $J_{20,22}$=10.5, $J_{9,8}$=11.5 resulting from the *trans* position of the vicinal protons.



24ξ-Ethyl-14β,22*R*-epithio-5α-cholestane (**1**, R=C_2H_5)

Figure 1.a) Carbon sequence established from inverse long-range ^1H-^{13}C correlation experiments
b) NOESY (Nuclear Overhauser effects)

^1H and ^1H-^{13}C long range NMR studies have been carried out on the mixture of the two C_{28} isomers, which could not be separated by HPLC. Although the NMR spectra of the mixture did not allow differentiation of the two isomers, it appeared clearly that both components have a 14β,22*R*-epithiosterane structure. The gas

chromatogram of the steranes obtained from Li/$EtNH_2$ reduction of the mixture of the two C_{28} steroid sulfides revealed the presence of two peaks consisting of 20*R*,5α(H),14α(H),17α(H)- and 20*R*,5α(H),14β(H),17α(H)-24-methylcholestanes[9] resulting from the non-specific reduction of position 14. However, as C-24 epimers are not separated under the GC conditions used,[10] it is likely therefore that the two C_{28} epithiosterane isomers correspond to epimers at position 24 (which are known to be extremely difficult to differentiate by NMR in the case of steroids). Similarly, the hydrocarbon skeletons of the C_{27} homologues could be deduced from the analysis of the products released by Li/$EtNH_2$ treatment which yielded 20*R*,5α(H),14α(H),17α(H)- and 20*R*,5α(H),14β(H),17α(H) stereoisomers of cholestane and 24-methyl-27-nor-cholestane, the latter being recently identified in sediments and coinjected with an authentic standard.[11]

The identification of this novel series of steroids as 14β,22*R*-epithiosteranes indicates that sulfur incorporation took place on steroids functionalized at positions 14 and 22. The presence of $\Delta^{8,14}$-sterenes has been reported in sediments such as the Monterey formation (California, USA)[6b], the Messinian Vena del Gesso formation (Italy)[12] or Gibellina samples (Sicily, Italy)[13]. Such components, bearing an additional double bond at position 22 (frequent in algal steroids) could be suitable precursors to explain the formation of this novel series of steroid sulfides. However, considering the low abundance of $\Delta^{8,14}$ steroids in living organisms, their occurrence in sediments is more likely to result from the isomerisation of Δ^{7}-steroids, the latter being present in many organisms of phytoplanktonic origin, such as green algae or diatoms[14]. Interestingly, diatoms and/or dinoflagellates have been proposed as precursors for the unusual 24-methyl-27-nor-cholestane.[11] Further confirmation for an algal origin of the C_{28} and C_{29} 14β,22*R*-epithiosterane precursors comes from the similarity of their stable carbon isotope composition ($\delta^{13}C$=-18.2 and -18.4‰ respectively)[14] with those of the algal-derived 5β(H)- and 5α(H)-cholestane ($\delta^{13}C$=-18.7 and -19.4‰ respectively) measured on the same sample and which are clearly distinct from the $\delta^{13}C$ values of higher plant components ($\delta^{13}C$ values between -25 and -29‰).

Table 1. ^{1}H and ^{13}C NMR assignments for 24ξ-ethyl-14β,22*R*-epithio-5α-cholestane (**1**, R=C_2H_5) in $CDCl_3$.

C	$\delta^{13}C$ ppm	$\delta^{1}H$ ppm		C	$\delta^{13}C$ ppm	$\delta^{1}H$ ppm	
1	38.68	1.67 (β)	0.86 (α)	**16**	21.11	1.74 (α)	1.58 (β)
2	22.18	1.49	1.38	**17**	54.07	1.45	
3	26.83	1.66	1.21	**18**	18.27	1.28	
4	28.96	1.15-1.27	1.15-1.27	**19**	12.22	0.77	
5	46.92	1.00		**20**	36.75	2.04	
6	29.18	1.23	1.15	**21**	18.89	0.89	
7	29.57	2.06 (β)	1.01 (α)	**22**	41.90	2.70	
8	37.83	1.57		**23**	35.17	1.67	1.30
9	48.79	1.08		**24**	42.54	1.27	
10	36.85			**25**	27.99	1.81	
11	19.81	1.49 (α)	1.33 (β)	**26**	20.48	0.88	
12	34.09	1.39 (α)	1.20 (β)	**27**	16.99	0.76	
13	45.71			**28**	22.49	1.38	0.97
14	61.43			**29**	12.52	0.87	
15	34.19	2.39 (α)	1.98 (β)				

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Germany; Geologisches Institut der Universität Köln, Germany; Laboratoire de Chimie Organique des Substances Naturelles associé au CNRS, Université Louis Pasteur, Strasbourg, France; Netherlands Institute for Sea Research, Department of Marine Biogeochemistry and Toxicology, Texel, the Netherlands; Department of Environmental Chemistry, CID-CSIC, Barcelona, Spain; Organic Geochemistry Unit, University of Bristol, U.K. and receives funding from the European Community (EC contract # CHRX-CT94-0474).

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15. $\delta^{13}C=10^3[(R_x-R_s/R_s)]$, where $R=^{13}C/^{12}C$, x: sample, s: PDB standard and $R_s=0.0112372$.

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