STEROID ACIDS IN PETROLEUM—ANIMAL CONTRIBUTION TO THE ORIGIN OF PETROLEUM

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ABSTRACT

The recent discovery of four steroid acids in a California petroleum is described. The observed ratios of *cis*- to *trans*-stereoisomers of the A/B rings of the steroid skeleton justify postulation of some animal contribution to the origin of this petroleum.

Mechanisms of formation from animal bile acids and unsaturated sterols are discussed. The conclusions are that all steroid acid isomers found can be explained from animal sources. Plant sources are likely contributors; however, they alone cannot explain all the isomers in their observed ratios.

INTRODUCTION

The original discovery of porphyrins in petroleum and sediments by Treibs[1] which triggered the theory that petroleum is of biological origin had two aspects:

(a) Proof of the presence of major portions of desoxophylloerythroetioporphyrin, which is chlorophyll—and, thus, plant—derived;

(b) The presence of a very small portion of mesoetioporphyrin was indicated spectroscopically. It belongs to the haeme family, animal haemoglobin being a possible precursor.

Corwin[2] discussed plant versus animal petroleum genesis chemotaxonomically, estimating that plant material would outweigh the animal by a factor of 100 000 to 1. Baker[3] commented 'this makes likely that minor pigments of the haeme type in the plant sources contribute more to the overall store of the petroporphyrins than the major pigments of animal origin', and Corwin[2] concluded 'a complete plant origin of the petroporphyrins is conceivable.'

It is the purpose of this brief paper to demonstrate how the interpretation of stereochemical details of some steroid acids recently discovered[4] in petroleum can possibly be utilized to shed light on such questions as an animal contribution to the abundant plant genesis of petroleum.

THE STRUCTURE OF THE STEROID ACIDS

Our interest in the structure of individual carboxylic acids in petroleum grew out of our work on classes[5a] of carboxylic acids in a virgin crude oil (Midway Sunset Field, California) of Pliocene age; it resulted in the addition

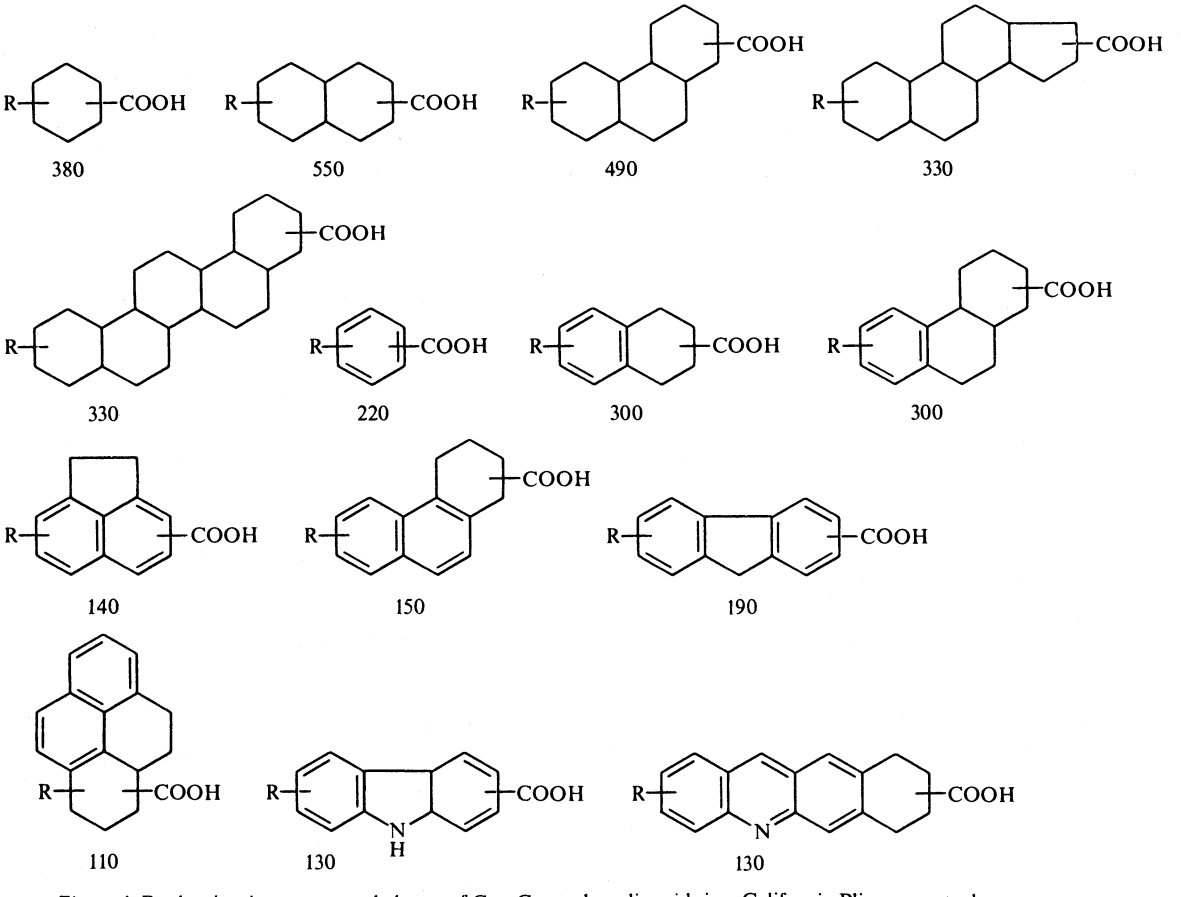
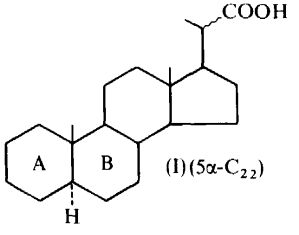


Figure 1. Predominating compound classes of C_{16}–C_{31} carboxylic acids in a California Pliocene petroleum

Values are given in ppm and are based on crude oil and represent minimum estimates.
Position of substituents, rings, and ring sizes unknown.

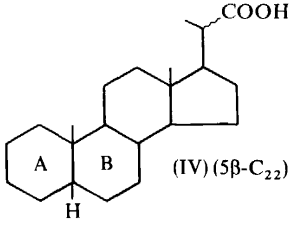
of some 40 new compound classes of petroleum carboxylic acids[5b] to the few known previously and the literature was summarized in one of our recent papers.[5c] *Figure 1*, which lists the predominant compound classes, illustrates how the polycyclic naphthenic acids outrank the others in relative abundance.

After reducing the enormous complexity of the mixture by extensive separation[5d], a small fraction (1.6 per cent of the acid fraction, 0.04 per cent of the petroleum) enriched in these classes was subjected to a detailed structural investigation via reductive deuterium labelling[4b] and esterification followed by gas chromatography plus mass spectrometry. In spite of the still considerable complexity (1500 estimated compounds[5e]), proof of structure was accomplished by synthesis for four individual steroid carboxylic acids, which occurred in considerably larger quantities than most of the other individual compounds[4b]. The most abundant species were two stereoisomers (at C_{20}) of 5α-pregnane-20ξ-carboxylic acid (I), for convenience



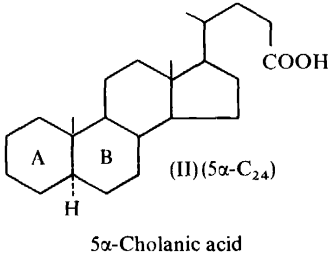
(I) (5α-C_{22})

5α-Pregnane-20ξ-carboxylic acid
A/B *trans*



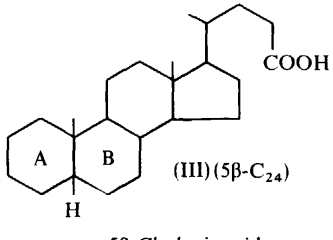
(IV) (5β-C_{22})

5β-Pregnane-20ξ-carboxylic acid
A/B *cis* (nearly absent)



(II) (5α-C_{24})

5α-Cholanic acid
A/B *trans*



(III) (5β-C_{24})

5β-Cholanic acid
A/B *cis*

referred to as 5α-C_{22}. Both possess A/B *trans*-configuration and form one and the same hydrocarbon after reduction. The other two acids found are 5α-cholanic acid, (II, 5α-C_{24}) and 5β-cholanic acid (III, 5β-C_{24}). The near absence of 5β-pregnane-20ξ-carboxylic acid (IV, 5β-C_{22}) was demonstrated previously by synthesis of the corresponding hydrocarbon and coinjection (Figure 2E of Reference 4b).

Recent investigation of an acid fraction representing a large portion of all carboxylic acids (namely, 40 per cent, equal to 1.1 per cent of the petroleum), by reduction to hydrocarbons, triple silica gel, and alumina chromatographic isolation of all saturates and subsequent GC separation plus coinjection with

synthetic steroid hydrocarbons, confirmed the ratios of steroid acids previously obtained by monitoring the parent ions of their esters (Figure 4 of Reference 4b) except for one difference: the amount of 5β-C_{24} as determined by GC coinjection was found to be larger than its 5α-C_{24} stereoisomer. The GC results were supported by gas chromatography mass spectrometry (GCMS) using the mass spectrometer as a detector and monitoring the predominating steroid fragment *m/e* 217. By both detector methods, the absence of 5β-C_{22} could again be ensured by coinjection with the synthetic 5β-C_{22} hydrocarbon. Both methods showed a ratio of 5α-C_{22}:5β-C_{24} of about unity (*Figure 2*): and, most important, both methods agreed analytically on the predominance of 5β-C_{24} over 5α-C_{24} (GC ~ 9:1, GCMS ~ 4:1).

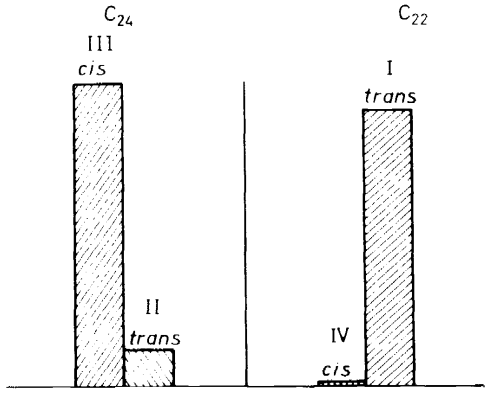


Figure 2. Relative ratios of stereoisomeric steroid acids. Semiquantitative, based on GC and GCMS of acid-derived hydrocarbons; rings A/B *cis* or *trans*

Quantitative differences between the two methods are easily explicable by a possible contribution of some other components to the 5β-C_{24} GC peak, sensitivity variations of the *m/e* 217 fragments as observed for the synthetic esters of the corresponding acids (Table II of Reference 4b), or a combination of these causes.

For chemotaxonomic purposes, the results obtained on the acid-derived saturated hydrocarbon fraction are preferred over those derived from smaller fractions because this fraction is representative of a large fraction of the acids (40 per cent) and of 1 per cent of the petroleum, and the mixture is less complex. As outlined in the following, the preliminary conclusions based on ester data[4b] remain unaffected.

In summary, the ratios of stereoisomeric steroid acids are: 5α-C_{24} (*trans*) < 5β-C_{24} (*cis*) ≅ 5α-C_{22} (*trans*) ≫ 5β-C_{22} (*cis*). Regardless of what the source is, thermodynamic equilibrium between both pairs (*trans*-C_{22}/*cis*-C_{22} and *trans*-C_{24}/*cis*-C_{24}) cannot have been reached because they are too different. In the C_{24} series, the thermodynamically less stable *cis*-isomer[6] predominates over its *trans*-counterpart. In the C_{22} series with proof of absence of C_{22}-*cis*, the situation is grossly reversed (*Figure 2*).

Furthermore, the marked predominance of 5β-C_{24} (*cis*) over 5β-C_{22} (*cis*) compared with the reversed predominance in the *trans*-series (5α-C_{22} > 5α-C_{24}, compare *Figure 2*) requires some rationalization.

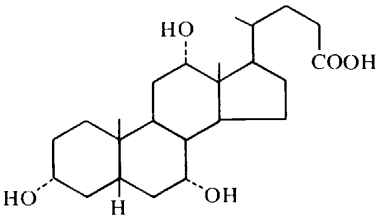
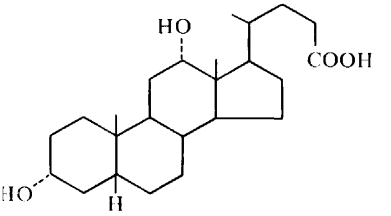
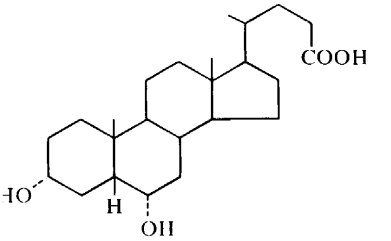
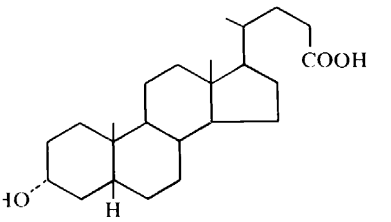
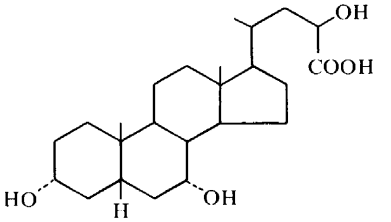
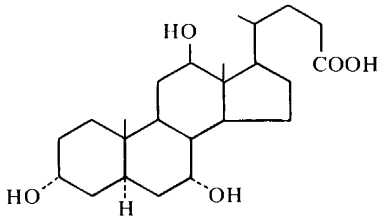
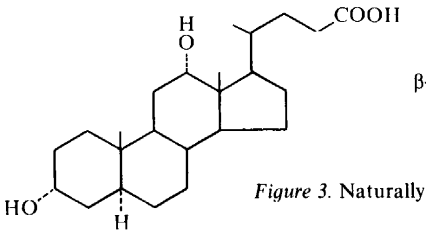
Structure	Acid	Representative sources
	Cholic	Bird, fish, snake, and others
	Desoxycholic	Ox, rabbit, and others
	Hyodesoxycholic	Hog, boar
	Lithocholic	Rabbit, hog, man
	Phocaecholic	Sea lion, walrus
	Allocholic	Lizard
	β-Lagodesoxycholic	Rabbit

Figure 3. Naturally occurring C_{24} bile acids

THE ORIGIN OF THE STEROID ACIDS

Animal origin. Figure 3 shows some examples of C_{24} animal bile acids occurring in amphibians and some lower invertebrates[7], most of which existed during Pliocene time. They all contain various numbers of hydroxyl groups. The A/B stereochemistry of most acids reported is *cis*. However, some *trans*-acids have been found in lizards and rabbits. *Figure 4* depicts how the formation of all the petroleum steroid acid isomers identified can easily be explained from these animal bile acid precursors. Dehydration and sub-

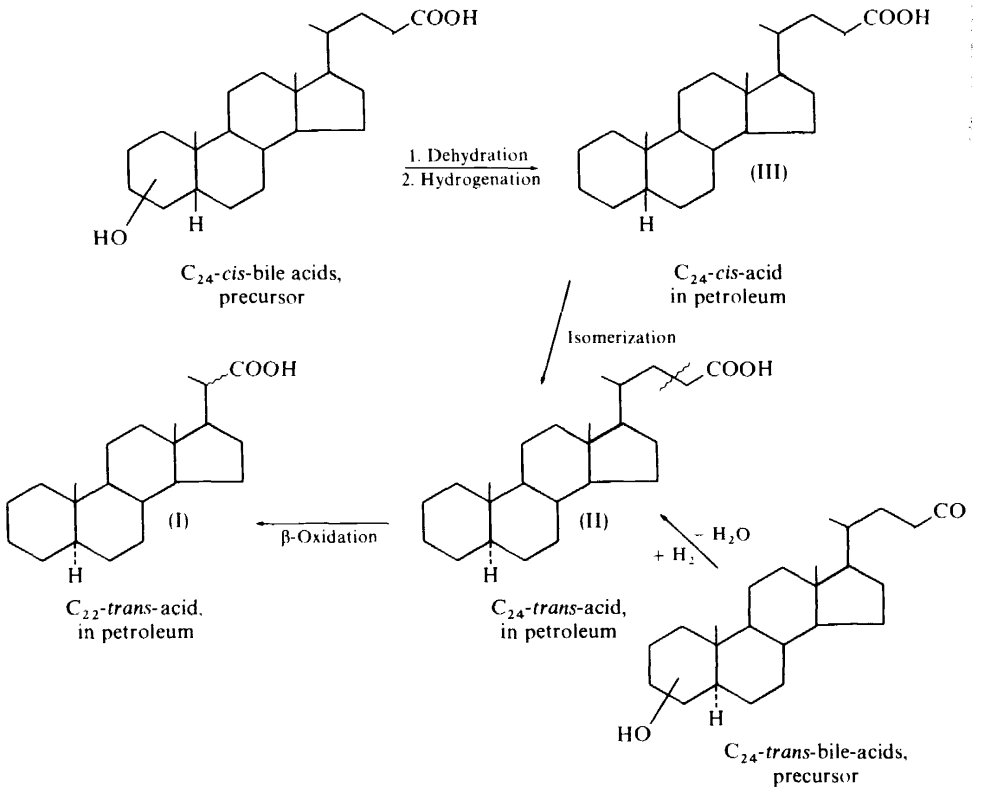


Figure 4. Possible animal origin of steroid acids

sequent hydrogenation of *cis*- and *trans*-bile acids is the chemically most facile route to the observed 5α- and 5β-cholanic acids (*trans*- and *cis*-C_{24}). Such concepts are generally accepted by petroleum chemists because minerals are good dehydration catalysts, and petroleum is a hydrogenating medium. Some isomerization of the thermodynamically less stable *cis*-compound, (III), to its *trans*-isomer, (II), is likely. Stereospecific β-oxidation of *trans*-C_{24}-acid, (II), to *trans*-C_{22}-acid, (I) (*Figure 4*), could explain the high concentration of (I) and low concentration of (II) (*Figure 2*).

Plant origin. The plant kingdom is abundant in sterols which could readily yield the C_{22}-*trans*-acid, (I). An example is dihydroergosterol (*Figure 5*).

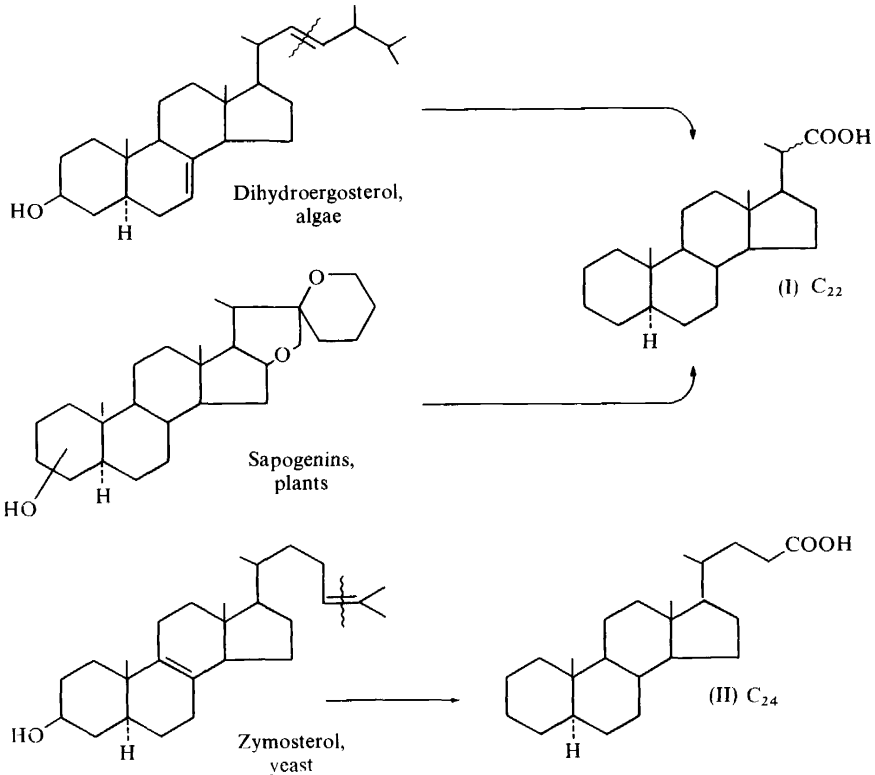


Figure 5. Possible plant origin of 5α-steroid acids (oxidation, dehydration, reduction)

Analogously, C_{24}-*trans*-acid, (II), can possibly be derived by oxidative C_{24}—C_{25} double-bond cleavage of zymosterol (*Figure 5*), the only sterol of this type noted in the literature. An alternative, though less likely, path to C_{22}-*trans*-acid (I) would begin with steroidal sapogenins (*Figure 5*). The fact that three C_{22}-steroid acid isomers, all of which possess A/B *trans*-configuration, were found[4b] reflects a variety of sources. Thus, 5α-C_{22} and 5α-C_{24} can be explained from plant sources; however, it fails for 5β-C_{24} (5β-cholanic acid) because of the lack of reported 5β-C_{24} steroids in the plant kingdom. Had 5β-C_{24} come from plant-derived 5α-C_{24} by isomerization of the latter, we would expect it to happen to a similar extent to 5α-C_{22}; and we would expect to find more 5β-C_{22} than 5β-C_{24} because there is more 5α-C_{22} than 5α-C_{24} (*Figure 2*). However, the reverse is the case in the hydrocarbon work proving the near absence of 5β-C_{22}. This absence of 5β-C_{22} is a rather important point and supports the conclusion that plants alone cannot be the sole source of 5β-cholanic acid.

Regarding the path of formation of the C_{22}-*trans*-acid, one has to weigh the ease of chemical transformation from bile acids (*Figure 4*) chemotaxonomically against the excess abundance of plant sterols over animal bile acids in nature (*Figure 4* and *Figure 5*).

CONCLUSIONS

The conclusion reached after consideration of all alternatives for each steroid acid isomer as well as their relative abundances is that all steroid acids can be explained from animal sources. Plant sources are likely contributors; however, they cannot be the sole precursor material.

At this point, one should remember that the steroid acids occurring in the ppm range based on total crude oil are a very small portion of the whole petroleum. Thus, the derivation from animal sources is still chemotaxonomically consistent with predominant plant diagenesis of this petroleum. However, the above outlined discussion is an example of how the recognition of intricate stereochemical details can raise the proof of structure of a new class of biologically derived compounds in petroleum beyond the merely descriptive stage and render it biogeochemically significant. It has to be conceded that the arguments presented in this paper only lead to a very likely hypothesis. Proof would require a demonstration of the absence of bacterial activity.

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