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Thus far, our search for molecular carbonaceous remnants, specifically indicative of biogenic processes, has been epitomized by the isolation and identification of isoprenoid alkanes in a number of ancient sediments of various geologic types ranging from several million to over two and a half billion years in age.[1,2,3] We have felt that the isoprenoid alkanes could serve as "biological markers" in our quest for evidence of life among geologically well-characterized ancient shales and oils, and more specifically in carbonaceous chondrites.

Earlier we reported preliminary studies on the oil shale from the Green River Formation (Eocene Age, about 60 x 10^6 years) at Rifle, Colorado.[1,2] These results, which paralled those of Cummings and Robinson,[4] established the biological history of this Cenozoic rock from the very uneven distribution of the n-alkanes and from the presence of large proportions of isoprenoid alkanes [C_{16}-, C_{18}-isoprenoid; C_{19}-isoprenoid (pristane); C_{20}-isoprenoid (phytane)].

We wish to report now the isolation and identification of the C_{27}-, C_{28}- and C_{29}-steranes and a C_{30}-penta-cyclic triterpane from the branched-cyclic alkane fraction of the Green River Shale.

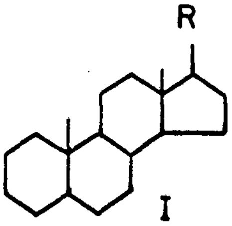
Separation of the branched-cyclic alkane fraction (extraction from shale previously described in detail$^{2)}$) into its individual components was achieved by an initial programmed gas-liquid chromatographic run to 300° (3% SE-30 on Gaschrom Z, 100-120 mesh, 10' x 1/4" s.s. column, program rate 4° per min.) in which peaks were collected and in turn rechromatographed isothermally at temperatures ranging from 260°-280° (1% SE-30 on Gaschrom Z, 100-120 mesh, 10' x 1/4" s.s. column). Mass spectra of these collected samples were determined in a direct inlet system of a modified C.E.C. 21-103C mass spectrometer.[5]

A preliminary mass spectral examination of the fractions collected as indicated in Fig. 1 from a programmed run revealed the molecular weights which are listed in Table 1 for the major components. From the subsequent isothermal separations, a homologous series of compounds were isolated which display mass spectral fragmentation patterns characteristic

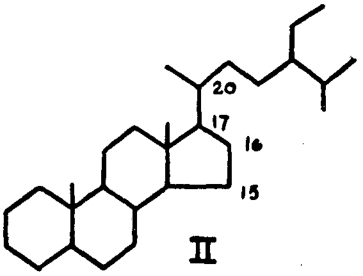
<u>Table 1</u>

Fraction a	372
b	386
c	386, 400
d	386, 400, 412
e	386, 400, 412
f	400, 412

of the saturated, tetracyclic sterane carbon skeleton (I). The mass spectra of the isolated C_{27}-, C_{28}- and C_{29}- steranes are shown in Figs. 2, 3 and 4, respectively, together with the mass spectrum[6] of authentic

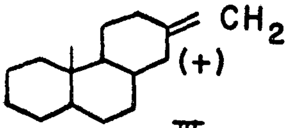


I



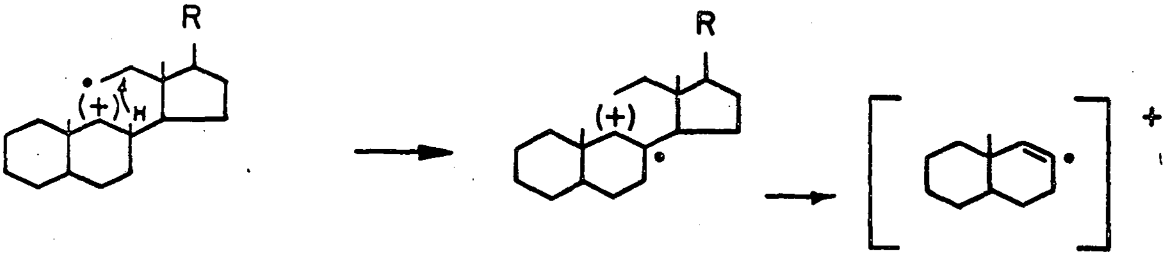
II

sitostane (II) in Fig. 5.[7] The intense peak of m/e 217, which is common to these spectra is depicted as III and arises through loss of the side



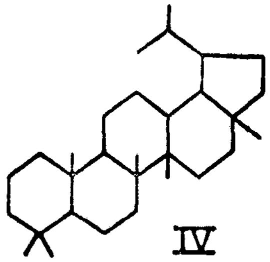
III

chain together with C-15, C-16 and C-17 of ring D and demonstrates that rings A, B and C are saturated and unsubstituted. Another very favorable mode of decomposition gives rise to the intense peak at m/e 149, as illustrated mechanistically by the following sequence:



The small peak at m/e 259 embodies the tetracyclic moiety resulting from simple cleavage of the 17,20-bond. Thus, the substitution causing the homology is present in the side chain. The mass spectrum of fraction d is shown in Fig. 6. The molecular weight of 412 demands five degrees of unsaturation, therefore, a penta-cyclic carbon skeleton.[8] The relatively large peak at m/e 369 (412-C_3H_7) in conjunction with peaks at m/e 123, 137, 191, 205 and 231 is indicative of a penta-cyclic triterpane in the lupeol series.[9]

The identification as a C_{30}-triterpane is corroborated by comparison of its fragmentation pattern (Fig. 6) with that of authentic lupane[10] (IV) in Fig. 7.



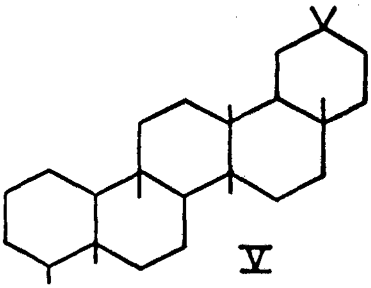
IV

The appearance of the C_{28}- and C_{29}-steranes in a number of different glc fractions (as indicated in Table 1) suggests the presence of several isomers of each homologue. Such a finding could be interpreted in terms of abiogenic reduction of their precursors, e.g., ergosterol, etc., resulting in different ring junctures (A/B cis versus A/B trans, etc.) and, therefore, isomers with different gas-chromatographic retention times, although a certain amount of overlap of glc peaks could account for their multiple presence. Further study is in progress to ascertain which stereoisomers are present in the Green River Shale.

The organic extract from shale of the Soudan Iron Formation of Minnesota which is the oldest carbonaceous rock thus far known on the North American continent and has been dated isotopically at greater than two and a half billion years[11] has resulted in the isolation and identification of a series of "molecular fossils", the isoprenoid alkanes.[3]

Concurrently, an analogous investigation of the branched-cyclic alkane fraction from the Soudan Shale (extraction procedures previously described in detail[2]) was separated into "low boiling" and "high boiling" (above 200°) cuts by an initial programmed gas-liquid chromatographic run to 300° (3% SE-30 on Gaschrom Z, 100-120 mesh, 10' x 1.4" s.s. column, program rate 4° per min.). During a separate chromatographic run, authentic samples of cholestane and squalane were co-injected with both the branched-cyclic fraction (see Fig. 8) and the subsequent "high boiling" cut. After knowing these retention times for cholestane and squalane, the high boiling cut was re-chromatographed isothermally at 225° and the samples collected for mass spectrometric analysis. Fraction 20 corresponded in retention time to that of cholestane (see Fig. 8) and yielded upon mass spectrometric analysis the mass spectrum depicted in Fig. 9.

The mass spectral fragmentation pattern (Fig. 9) of fraction 20 has molecular ions at m/e 372, 386 and 400 indicative of the presence of C_{27}-, C_{28}- and C_{29}- saturated, tetracyclic hydrocarbons. This spectrum displays various features which are characteristic of the carbon skeletons (I) of a homologous series of three saturated steranes. Each of these molecular ions displays equally large fragments at $M-CH_3$; e.g., m/e 357, 371 and 385. The intense groups of peaks at m/e 217, 218 and 219 arise from the major mode of fragmentation in the steroid hydrocarbons having side chains attached to C-17, *vide supra*. It should be mentioned that there are several minor components of higher molecular weight, e.g., 410, 412, 414, 416 and 418, which contribute peaks to the fragmentation pattern of fraction 20. Many of these are peaks known to occur in the mass spectra of various triterpanes, e.g., 123, 137, 163, 177, 191, 231, etc., and consideration of the relative intensities of the appropriate peaks in fraction 20 is reminiscent of the mass spectrum of authentic friedelane (V).



V

Further work is in progress on the isolation and identification of C_{30} compounds in the triterpane series.

It is interesting to note the striking predominance of the sterane, penta-cyclic triterpane constituents in the branched-cyclic alkane fraction of the non-marine Green River Shale, particularly of those derived from the parent plant sterols. This contrasts with the much lower relative abundance of these compound classes in the Soudan Shale, which is of marine origin and much greater age. It is noteworthy that the probable diagenetic precursors of geologic steranes and triterpanes, e.g., ergosterol, sitosterol, lupeol, etc., occur in nature as allylic alcohols requiring geologic or bacterial reduction in a fashion analogous to that suggested for the conversion of phytol to phytane under geologic conditions.[12] Oxidizing conditions would yield keto steroids, but diagenesis involving sequential formation of a carboxylic acid and decarboxylation analogous to that presented for conversion of phytol to pristane and the C_{18}-isoprenoid alkane would be prevented in much the same manner that the formation of the C_{17}-isoprenoid alkane from phytol would be prevented. The C_{17}-isoprenoid alkane has not been isolated from ancient carbonaceous sediments thus far.[3,13]

Barton and co-workers[14] have identified oxyallobetul-2-ene, a derivative of a plant triterpenoid, from petroleum. In a series of papers Sorm and co-workers[15] have isolated and identified oxyallobetulone and several other triterpenoids from North Bohemian Brown Coal, the age of which is estimated to be tens of millions of years based on geological strata. Several reports have suggested the probable presence of steroid-type hydrocarbons in petroleum[16,17] and recent sediments.[18] Meinschein has indicated the presence of a C_{27}-sterane in the Nonesuch Shale on the basis of large peaks at 372, 218, 217 and 149 in the mass spectrum of a carbon tetrachloride eluant fraction from an alumina column.[19]

The greatly decreased solubility properties of steranes and triterpanes compared to the isoprenoid alkanes lends further support to evidence for the indigenous nature of these sedimental alkanes, rather than migration since sediment deposition.[3]

The occurrence of steranes (and probably penta-cyclic triterpanes) in the Soudan Shale provides further evidence for the presence of life processes sufficiently complex to require an enzymatic template and <u>in vivo</u> polyisoprenoid cyclizations at the two billion year mark in terrestrial chronology.

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[7] In Figs. 2, 3 and 4, peaks at m/e 191, 203 and 231 probably arise from small amounts of terpane impurities.

[8] This does not appear to be the same compound, molecular weight 412, reported by Cummings and Robinson[4] since the mass spectral fragmentation pattern is quite different. Personal communication, W. E. Robinson and J. J. Cummings, August 1965.

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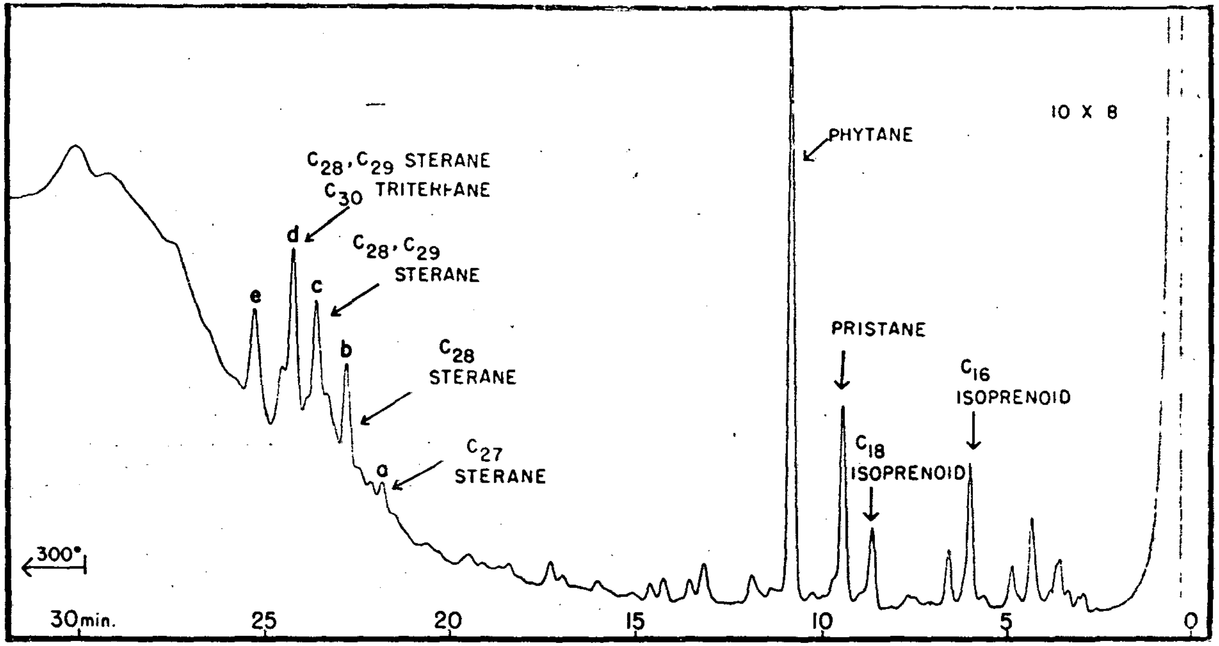
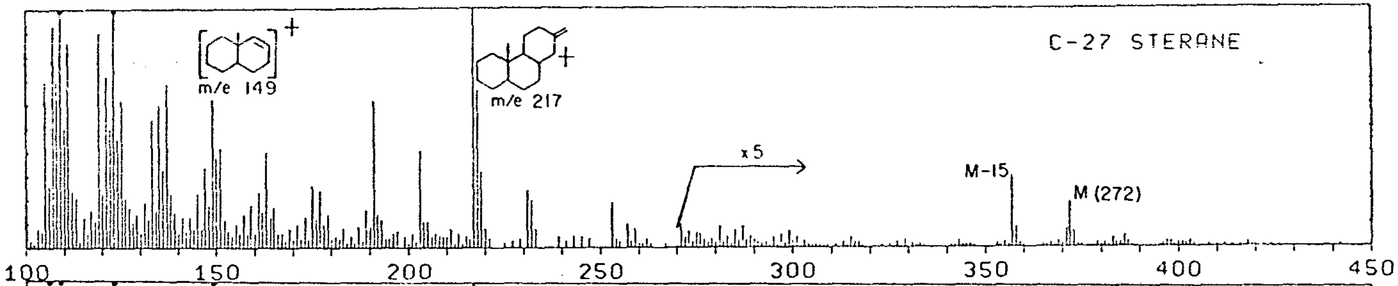
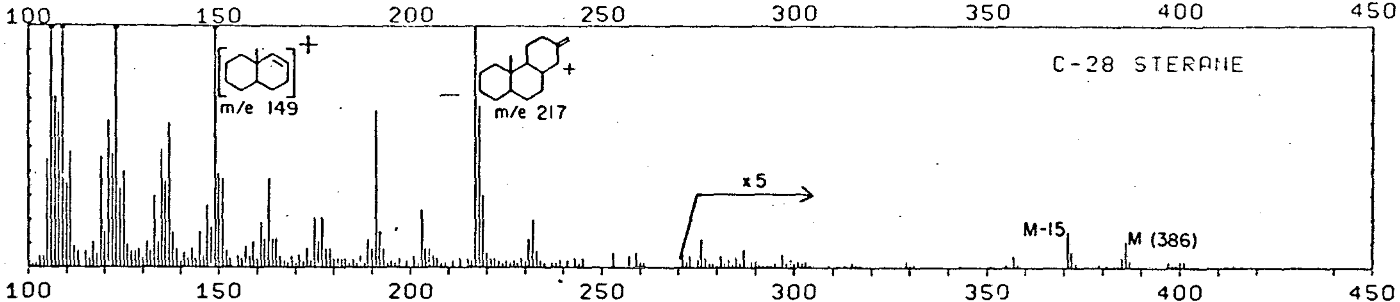
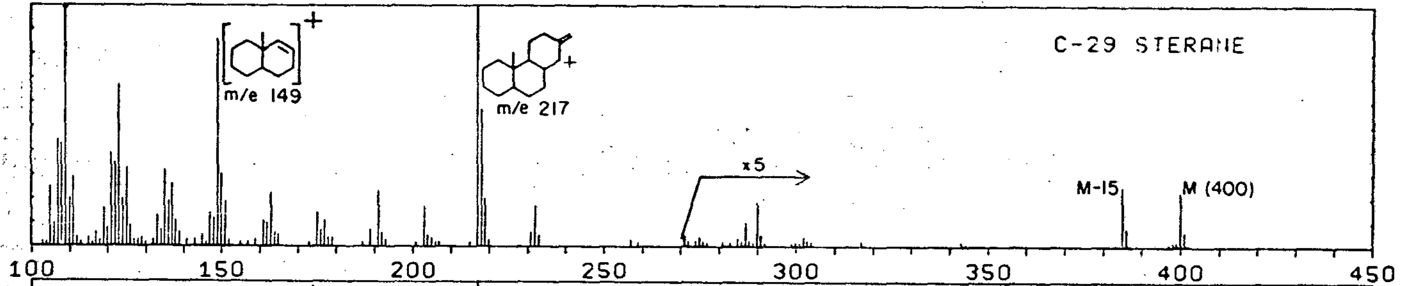


Fig. 1. Gas chromatogram of Colorado Green River Shale branched-cyclic alkane fraction







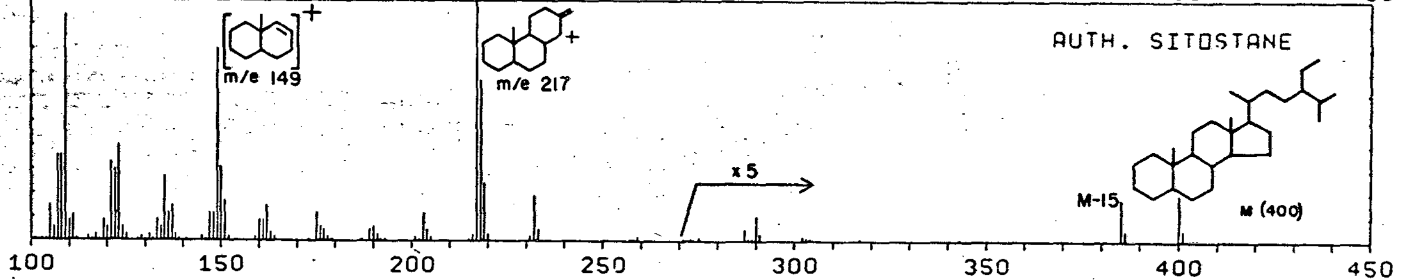
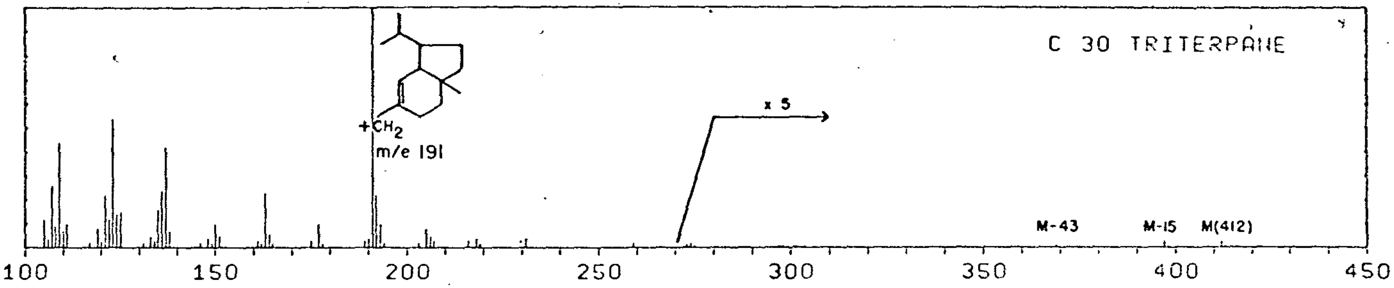


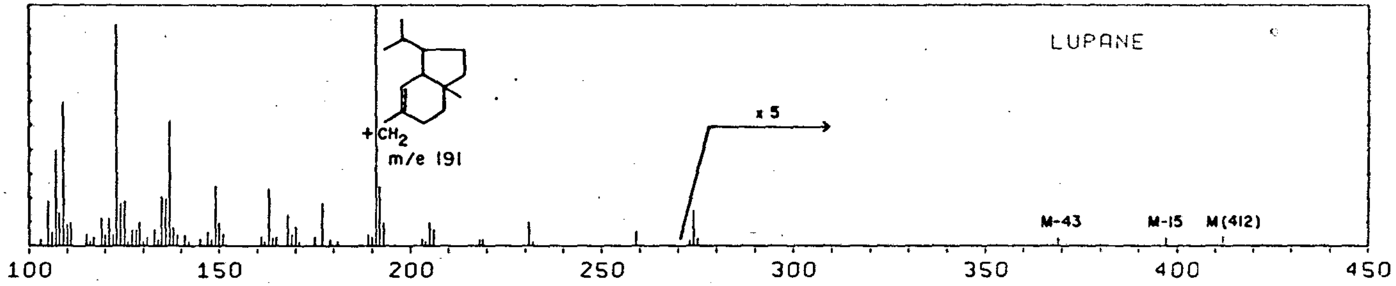
Fig. 2. Mass spectrum of C_{27} sterane

Fig. 3. Mass spectrum of C_{28} sterane

Fig. 4. Mass spectrum of C_{29} sterane

Fig. 5. Mass spectrum of sitostane





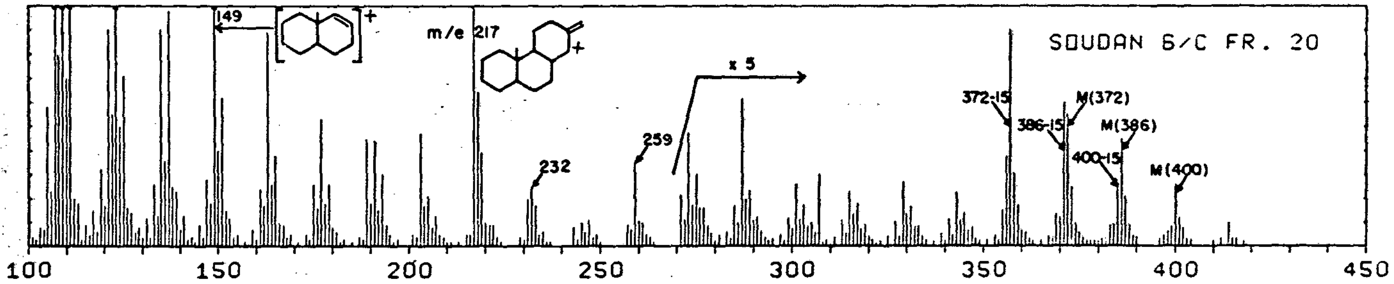


Fig. 6. Mass spectrum of C_{30} triterpane

Fig. 7. Mass spectrum of lupane

Fig. 9. Mass spectrum of Soudan Fraction 20

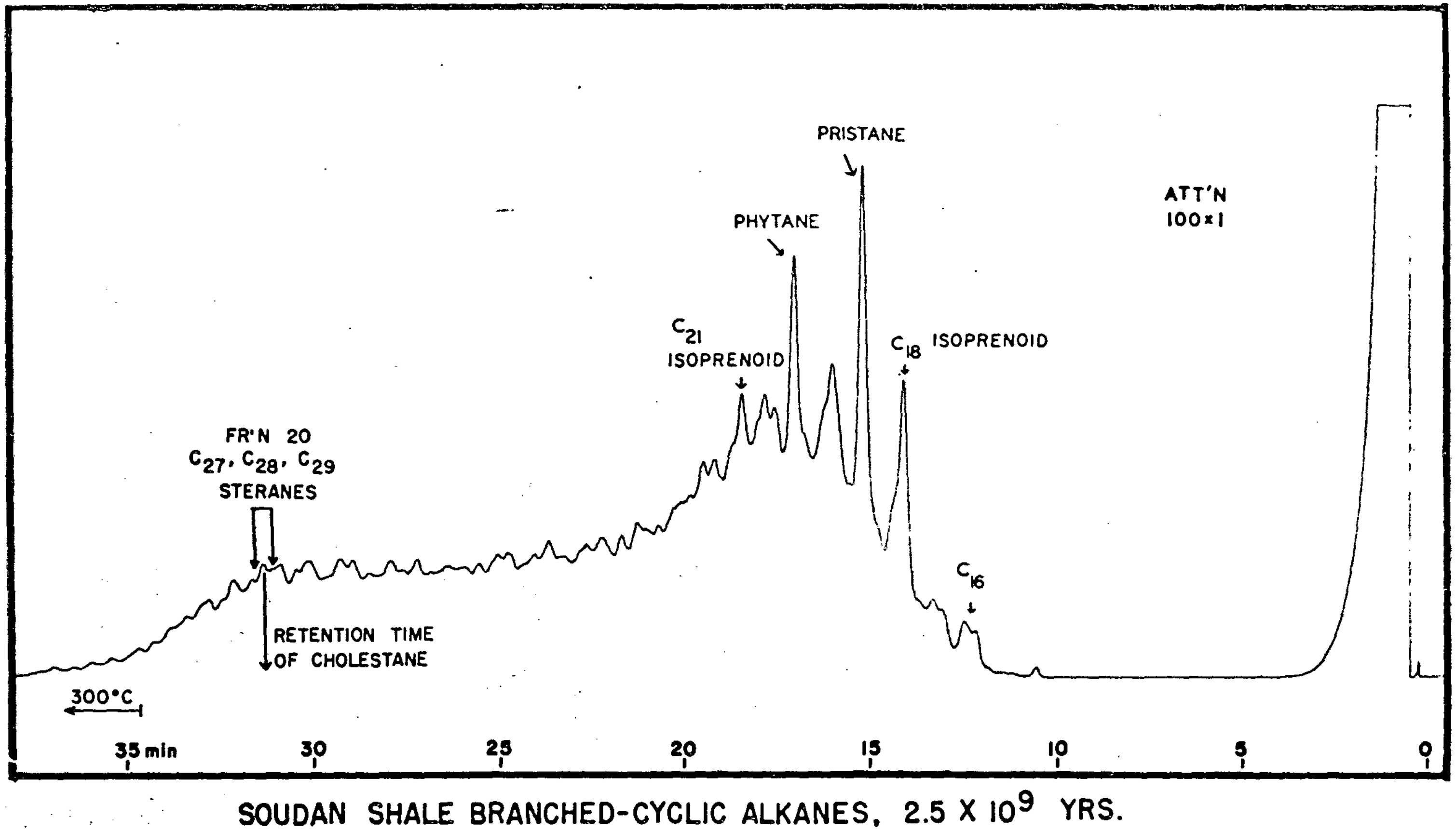


Fig. 8. Gas chromatogram of Soudan Shale branched-cyclic fraction