the resolution factors, confirm this view. However, the range of the differences in free energy of solution of the enantiomers (at 25°C), as calculated from the r values, is only 15 to 40 cal/mole. Conceivably, a somewhat different pattern could emerge under other conditions of comparison (*4*).

The lack of chiral differentiation by uridine (*5*) is in keeping with the lower stability of complexes of pyrimidines with polyaromatic hydrocarbons, as compared with purines (*6*). For the derivatives of adenine (**1** to **5**), any change made in the ribose molecule affects the r values. Thus, the resolution factors are somewhat reduced with respect to those of adenosine when the C_2' carbon acquires a symmetric configuration as in deoxyadenosine. Phosphorylation, as in 3′-AMP (**3**) 5′-AMP (**4**) and in cyclic AMP (**5**) affects the resolution in one sense or the other. The highest r values among the five adenine derivatives were obtained with 5′-AMP. Molecular rigidity can be favorable for chiral recognition, and there is less rotational freedom in 5′-nucleotides than in 3′-nucleotides and in nucleosides (*7*). Further modifications of the ribose moiety might be helpful in understanding the stereoselective phenomena we observed. The influence of the configuration at C_1' and the resulting change of the complexed helicene disposition could be studied by testing corresponding α-analogs.

The present and previous observations (*2*) on chiral differentiation of helicenes can be viewed in relation to the data on the metabolites of polyaromatic hydrocarbons on TAPA (*8*). The diols and diol-epoxides of benz[*a*]pyrene and benz[*a*]anthracene are assumed to play an important role in mutagenic and carcinogenic processes through covalent interaction with nucleotides, and their action is enantioselective (*9*). The stereoselectivity manifested by TAPA for metabolites of helicenes and polyaromatic hydrocarbons leads to the suggestion, by extrapolation of our data, that nucleic acids also form stereospecific complexes with these metabolites. This chiral differentation could be relevant to the enantioselective physiological effect.

YOUNG HWAN KIM
A. TISHBEE, E. GIL-AV
Department of Organic Chemistry, Weizmann Institute of Science, Rehovot, Israel

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Squalene in Petroleum Asphaltenes

Abstract. *Squalene, 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene, a new biological marker, has been detected in various petroleum asphaltenes and their pyrolysis products. Since squalene is the most common triterpenoid in nature and the biological precursor of the important tetra- and pentacyclic triterpane markers, its detection in asphaltene suggests new ways for marker correlations.*

Biological markers are molecules, produced by living organisms, with a molecular skeleton that has not been altered significantly. The presence of markers in petroleum and other fossil organic deposits can shed light on the nature of the source material and the genetic history of the deposits, and they provide proof of the biological origin of the deposits (*1*, *2*). Markers are also increasingly used in correlating reservoir oils with one another and with source kerogen and bitumen (*3*, *4*).

The common biological markers are all highly stable molecules, acyclic and cycloalkanes, aromatics, and their functionalized derivatives. Olefinic-type markers in petroleum are rare (*5–8*) and although squalene has been found in recent sediments, its presence has been regarded as an indication of in situ biomass (*9*).

Asphaltene, the heaviest high-molecular-weight fraction of petroleum, contains chemically bound markers (*10*), steranes and hopanes, in a similar yet characteristically different distribution from those of the saturate fraction of the oil. The main difference is less conversion of biological to nonbiological stereochemical types of steranes and terpanes in the asphaltene. This was attributed to the protective environment provided by the large, polymeric asphaltene molecules effectively preventing the trapped labile molecules from getting into contact with reagents, surface catalysts, or microbes capable of altering their chemical identity and leading to their destruction. A similar effect has been noted for kerogen compared with the associated bitumen (*4*). The importance of asphaltene as a source of geochemical information is now accentuated by the discovery of the presence of squalene, 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene, in asphaltene.

Squalene is the most common triterpenoid molecule in all life forms (*2*) and is the biological precursor of polycyclic triterpenoids in both plants and animals and, consequently, the precursor of the important series of biomarkers, the steranes and hopanes (*2*).

We found that asphaltenes from the Alberta oil sands, Utah oil sands, and from conventional crude oils contain squalene, which can be liberated by heat treatment (~ 300°C for 2 to 72 hours) under flow or static conditions. Mild thermolysis of Athabasca asphaltene produces a 10 percent yield (2 hours) of a pentane-soluble oil containing a saturate, monoaromatic, diaromatic, triaromatic, and resin fraction when analyzed by the procedure of Sawatzky *et al.* (*11*). The gas chromatogram of the diaromatic fraction featured a broad continuum with a superimposed large, sharp peak. Preliminary analysis of this fraction by gas chromatography–mass spectrometry gave the peak a mass of 410. The unknown material was then prepared in larger quantities by pyrolysis of Athabasca asphaltene and extraction with *n*-pentane. This pentane extract was subsequently fractionated by elution chromatography on a silica-alumina column to separate the diaromatic fraction (*11*). The desired peak was concentrated by high-performance liquid chromatography (HPLC) on μ-Porasil columns with hexane used as eluent. The molecular weight of the purified material was determined by high-resolution mass spectrometry, which gave a value of 410.3921, identifying the unknown material as a $C_{30}H_{50}$ hydrocarbon (theoretical value, 410.3913). The molecular weight of an authentic squalene sample was 410.3909. Further identification was then achieved by comparison with an authentic sample of squalene by (i) gas chromatography retention times on two different columns

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Table 1. Squalene content of the pyrolyzate of asphaltenes and of some microorganisms.

Asphaltene source	Type of oil	Depth (m)	Squalene (ppm) lower limit
Tar sands and heavy oils			
Athabasca	TS*	10	200
Peace River	HO†	570	170
Cold lake (Leming Lake)	HO	370	175
Cold lake (Marguerite Lake)	HO	370	50
Lloydminster	HO	670	25
Utah Sunnyside	TS	Surface	40
Utah asphalt ridge	TS	Surface	170
Conventional oils			
Triassic		1005	≥27
Upper Devonian		1544	≥18
Microorganisms			
Yeast (brewers' debittered)			600
Yellow algae (*Anabaena flos-aquae*)			12
Blue-green algae (Smoky Lake bloom) *Aphanizomenon*			2

*TS, tar sand. †HO, heavy oil.

(120- by 1/8-inch stainless steel, packed with 3 percent Dexsil 300 on Anachrom 90/100, and a 60-m OV-101 HF-etched capillary); (ii) relative mobility (R_f) values on a (silica gel) thin-layer chromatographic plate; (iii) detailed nuclear magnetic resonance spectrum; and (iv) mass spectrum. The mass spectrum was obtained on the HPLC eluent of an Athabasca asphaltene sample (Fig. 1).

Since various microorganisms produce squalene, a yeast and two algae samples were thermolyzed by methods similar to those used for the asphaltene samples, and the squalene content of the pyrolyzate was determined (Table 1).

A small amount of squalene can also be extracted from the natural Athabasca asphaltene by exhaustive pentane extraction with subsequent class analysis and gas chromatography of the diaromatic fraction. However, release of the major portion of the squalene requires heating of the asphaltene. This suggests that some of the squalene in the asphaltene is present in a chemically bound form. On thermolysis, the squalene may be liberated by means of a free radical mechanism in which a fraction of the $C_{30}H_{49}$ radicals are stabilized by hydrogen transfer as squalene, while the rest may be lost in other reactions. A search for squalene in the maltene- or pentane-soluble portion of the Athabasca bitumen revealed only trace quantities.

It is likely that at the time of asphaltene formation, squalene was present in the whole oil, but only those molecules incorporated into the asphaltene micelle survived. Since asphaltene, at least in the conventional oil field at Prudhoe Bay and the tar sand deposit at Athabasca (*10*), was formed at a relatively immature diagenetic age of the oil—as manifested by evidence of less conversion of biological to nonbiological stereochemical types of chemically bound steranes and terpanes in the asphaltene (*10*)—it may be assumed that squalene is not of recent origin, but belongs to the class of rather sensitive biological compounds that was protected from destruction. Under these circumstances it may be a constituent of the original organic portion of the sediment from which the oil was formed. The alternative hypothesis, that squalene arose from the bacteria responsible for the microbial degradation of the oil, appears less likely as a result of investigations on recent sediments (*9*). Since all of

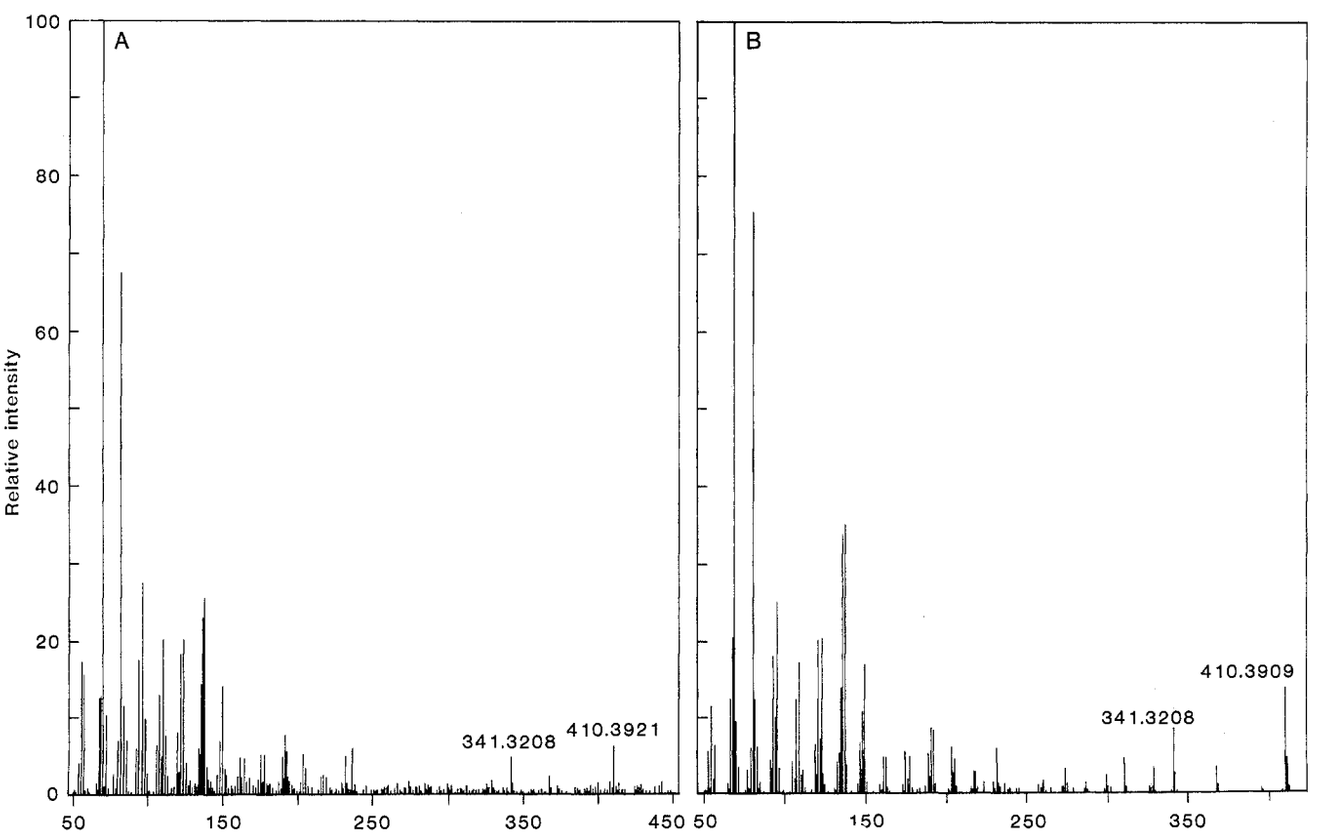

Fig. 1. Mass spectra of (A) the HPLC effluent of an Athabasca asphaltene sample and (B) of a commercial grade squalene sample.

the oil asphaltenes that we have examined so far yield squalene on mild pyrolysis, including those showing no sign of microbial degradation, the argument that this squalene is part of the original organic portion of the sediment appears to be strengthened. If so, then squalene must also be present in kerogens. The recent finding of squalene in Norwegian rocks supports this view (*12*). Since squalene is the biosynthetic precursor of plant steroids, it may also occur in trace amounts in coal.

All triterpenoids in nature are biosynthesized from squalene or from squalene-2-epoxide (*13*), and the chemically bound squalene found in asphaltene may be the squalene ether formed from squalene-2-epoxide. Experiments with added reagents known to promote carbon-oxygen bond cleavages in ethers, however, did not increase the squalene yields.

In conclusion, the widespread occurrence of squalene in petroleum asphaltene is a clear indication that squalene was a significant constituent of the biomass from which the oil formed and, as a new biological marker, will have practical application in oil correlations.

N. SAMMAN*
T. IGNASIAK, C.-J. CHEN
O. P. STRAUSZ, D. S. MONTGOMERY
Hydrocarbon Research Centre, Department of Chemistry, University of Alberta, Edmonton T6G 2G2, Canada

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* Present address: Gulf Canada Ltd., Research and Development Department, 2489 North Sheridan Way, Sheridan Park, Ontario L5K 1A8, Canada.

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Phosphorylation of Smooth Muscle Myosin: Evidence for Cooperativity Between the Myosin Heads

Abstract. *The relationship between the actin-activated adenosinetriphosphatase activity of smooth muscle myosin and the extent of myosin light chain phosphorylation is nonlinear. It is suggested that the phosphorylation of the two heads of smooth muscle myosin is an ordered process and that the two heads are influenced by cooperative interactions.*

The mechanism by which Ca^{2+} controls the activity of the contractile apparatus of smooth muscle is a controversial subject. The most popular theory is that the contractile activity is determined by the state of phosphorylation of two of the myosin light chains (*1*). Phosphorylation allows the activation by actin of the Mg^{2+}-adenosinetriphosphatase (Mg^{2+}-ATPase) activity of myosin and is thought to be a prerequisite for contraction; dephosphorylation of the myosin light chains eliminates the actin activation and therefore favors the relaxed state. The enzymes that are responsible for the phosphorylation and dephosphorylation have been characterized and are the myosin light chain kinase (*2*) and myosin light chain phosphatases (*3*), respectively. The alternative and minority viewpoint, held by Ebashi and co-workers (*4*), is that myosin phosphorylation is not significant and that regulation is achieved by a system based on the thin filaments, termed leiotonin. Whether these two mechanisms coexist within a single cell, are exclusive of each other, or are in some way complementary, is not known. The relative merits and contributions of each system must be established before the regulatory mechanism in smooth muscle is understood.

Our approach was to try to resolve the contribution made by one of the mechanisms above, namely phosphorylation. The relationship between the extent of myosin phosphorylation and the degree of activation by actin of the Mg^{2+}-ATPase activity has not previously been examined in a defined system, that is, one where each of the component proteins was separately purified and characterized. This is necessary to reduce the possible influence of unknown contaminant proteins. In particular, we wished to establish whether phosphorylation alone is sufficient for actin activation of ATPase activity and, if so, the relationship between the extent of phosphorylation and the level of ATPase activation.

The relationship between the extent of light chain phosphorylation and the activation of Mg^{2+}-ATPase activity by skeletal muscle actin is shown in Fig. 1. These data were collected by two procedures. In one method the time courses of myosin phosphorylation and adenosine triphosphate (ATP) hydrolysis were followed and at various times (4 to 10 minutes after the addition of ATP) the levels of phosphorylation were correlated to the ATPase rate, which was estimated from a tangent drawn to the relevant time point. In the other method myosin phosphorylation and ATPase activity were estimated from a single point at 10 minutes. The latter method is less accurate in that it could be affected by nonlinearity of either the phosphorylation or the ATPase reaction. However, use of the more convenient procedure is justified since both methods gave similar values, as shown in Fig. 1.

Figure 1 shows that the ATPase activity is not directly proportional to the degree of phosphorylation. Initially about 50 percent of the total sites are phosphorylated, and this results in only a slight activation of ATPase activity. At 50 percent phosphorylation the specific actin-activated ATPase activity is about 3 nmole of inorganic phosphate (P_i) liberated per minute per milligram of myosin. Subsequently, the remaining sites are phosphorylated and the ATPase activity is markedly enhanced. At saturating levels of phosphorylation the specific actin-activated ATPase activity is estimated to be about 28 nmole min^{-1} mg^{-1}. It appears, therefore, that significant activation of ATPase activity occurs only at relatively high levels of phosphorylation (> 50 percent), below which the ATPase activity does not show as marked a dependence on the degree of phosphorylation.

What this type of relationship means at a molecular level is worthy of some speculation. If phosphorylation of one of the two myosin heads resulted in ATPase activation of either that head or both heads, then a linear relation between ATPase activity and phosphorylation would be observed. This situation does not apply to the experimental data. Thus it can be suggested that myosin with only one head phosphorylated is not activated significantly by actin. Assuming that both heads contain equivalent enzymatic sites, it appears that the nonphosphorylated head inhibits the activity of the phosphorylated head or that an

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