choline. Our findings could also explain why, in shock lung syndrome, patients do not always respond to therapy with certain vasodilator drugs (*3*).

Irrespective of the exact mechanism, selective removal of endothelium from intrapulmonary arteries can transform two important circulating dilator agents into pulmonary vasoconstrictors. It is thus conceivable that damage in vivo to intrapulmonary arterial and arteriolar endothelial cells or destruction of pulmonary arterial endothelial cells by a number of agencies acting alone or in concert (for example, chronic hypoxia, release of hydrolytic enzymes, leukocyte platelet–endothelial cell interactions resulting in release of lysosomal enzymes, microaggregation of formed elements adjacent to these endothelial cells) may represent the major pathway in producing pulmonary hypertension or shock lung.

Our data may also explain why, in numerous studies on isolated pulmonary blood vessels—which are often traumatized in preparation—acetylcholine and certain other so-called vasodilators will produce contraction rather than dilation of these vessels (*6*).

NARESH CHAND
BURTON M. ALTURA
Department of Physiology, State University of New York, Downstate Medical Center, Brooklyn 11203

References and Notes

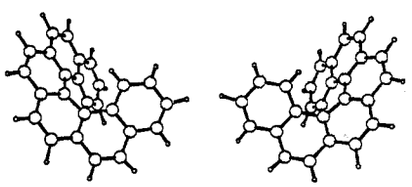
1. R. F. Furchgott and J. V. Zawadzki, *Pharmacologist* **21**, 271 (1979); *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 581 (1980); *Nature (London)* **288**, 373 (1980); ———, P. D. Cherry, in *Mechanisms of Vasodilatation*, P. M. Vanhoutte and I. Leusen, Eds. (Raven, New York, in press), vol. 2.
2. J. B. West, *Pulmonary Pathophysiology—The Essentials* (Williams & Wilkins, Baltimore, 1977); J. K. Perloff, in *Pulmonary Vascular Disease*, K. M. Moser, Ed. (Dekker, New York, 1979), p. 489; M. Voelkel and J. T. Reeves, in *ibid.*, p. 573.
3. A. P. Thal, E. B. Brown, Jr., A. S. Hermreck, H. M. Bell, *Shock. A Physiologic Basis for Treatment* (Year Book, Chicago, 1971); J. W. Wilson, in *The Cell in Shock* (Upjohn, Kalamazoo, Mich., 1974), p. 39; B. J. Pardy and H. A. F. Dudley, *Surg. Gynecol. Obstet.* **144**, 259 (1977).
4. N. Chand and B. M. Altura, *J. Appl. Physiol.* **49**, 1016 (1980); *Prostagland. Med.* **5**, 59 (1980); B. M. Altura and N. Chand, *Br. J. Pharmacol.*, in press.
5. B. M. Altura and B. T. Altura, *Am. J. Physiol.* **219**, 1698 (1970).
6. E. H. Bergofsky, in *Pulmonary Vascular Disease*, K. M. Moser, Ed. (Dekker, New York, 1979), p. 233; P. M. Vanhoutte, in *Microcirculation*, G. Kaley and B. M. Altura, Eds. (University Park Press, Baltimore, 1978), vol. 2, p. 181.
7. Intrapulmonary arteries were fixed in 2 percent glutaraldehyde and 0.1*M* potassium phosphate (*p*H 7.4) for 60 minutes at 4°C, postfixed in 1 percent O_sO_4 and 0.1*M* potassium phosphate, dehydrated in a graded ethanol series, and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate. These were then examined and photographed in a JEOL 100C electron microscope.
8. Supported in part by PHS research grants NHLBI 18002, NHLBI 18015, and DA 02339 (to B.M.A.). We thank G. K. Ojakian and D. Herzlinger for performing the electron microscopy. Requests for reprints should be addressed to B.M.A.

16 April 1981; revised 4 June 1981

Chiral Recognition by Nucleosides and Nucleotides: Resolution of Helicenes by High-Performance Liquid Chromatography

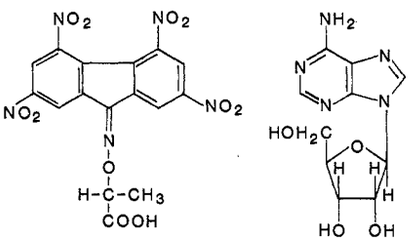
Abstract. *Chiral recognition by nucleosides and nucleotides coated on silica gel was studied by high-performance liquid chromatography. Helicenes, which are chiral polyaromatic hydrocarbons, were used as probes. Stereoselectivity was detected when the nucleobase was a purine (adenosine, deoxyadenosine, adenosine 3′-monophosphate, adenosine 5′-monophosphate, adenosine 3′,5′-monophosphate, and guanosine), but was not detected with the pyrimidine derivative uridine. For a given nucleobase (adenine), all changes in the ribose moiety affected the resolution factors, which ranged between 1.03 and 1.074. These results might be relevant to the enantioselectivity of carcinogenic metabolites of polyaromatic hydrocarbons.*

We recently described enantioselective interactions of riboflavin with chiral ortho-condensed polyaromatic hydrocarbons as manifested in resolutions of optical isomers by high-performance liquid chromatography (HPLC) (*1*). We now report a similar study with nucleosides and nucleotides coated on the column packing material. As in the riboflavin study (*1*), helicenes, which have a helical shape due to overcrowding, were the compounds resolved. Although helicenes do not occur in nature, the results may be of interest for probing the capacity of nucleic acid building blocks for chiral differentiation.



P(+)-Hexahelicene M(−)-Hexahelicene

The compounds tested were **1**, adenosine; **2**, deoxyadenosine; **3**, adenosine 3′-monophosphate (3′-AMP); **4**, adenosine 5′-monophosphate (5′-AMP); **5**, adenosine 3′,5′-monophosphate (cyclic AMP); **6**, guanosine; and **7**, uridine. Purines and pyrimidines form complexes with polyaromatic hydrocarbons. Combination of a purine or pyrimidine with a chiral substituent such as ribose could lead to chiral differentiation of optically active polyaromatic hydrocarbons, in analogy



R(−)-TAPA Adenosine

with the behavior of *R*(−)-2-(2,4,5,7-tetranitrofluorylidene-9-aminooxy)propionic acid (TAPA) (*2*) and of riboflavin (*1*). The molecular interaction of nucleobases with polyaromatic hydrocarbons differs from that of TAPA and riboflavin, since for the nucleobases the contribution of charge transfer is considered to be of little importance as compared with van der Waals forces (*3*).

The nucleosides and nucleotides were coated on 5-μm silica gel (Lichrosorb Si 100, Merck, Darmstadt, Germany) and HPLC columns were prepared by slurry packing. The amount of coated material, determined by elementary analysis, var-

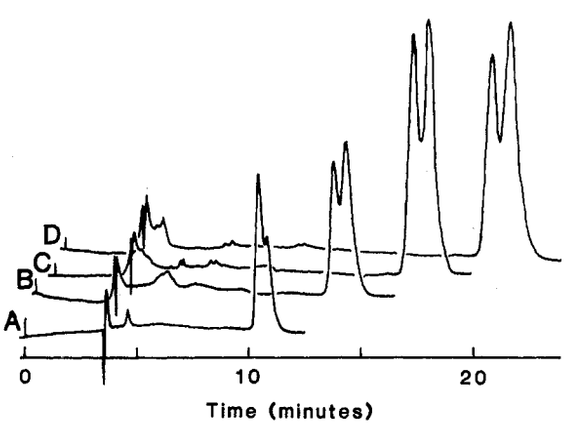


Fig. 1. Resolution of the optical isomers of [10]- to [13]-carbohelicenes on adenosine-coated silica gel. The number in brackets indicates the number of rings in an individual helicene. The chromatographic system consisted of a Waters 6000A pump, a Reodyne 7120 injector with a 20-μl loop, and an LDC ultraviolet monitor set at 254 nm. The column was 20 by 0.46 cm (inside diameter). The eluant was CH_2Cl_2 and *n*-hexane (1:9); the flow rate was 1 ml/min; and the temperature 23° to 25°C. The elution curves correspond to (A) [10]-helicene enriched in the *M*(−)isomer, and (B) [11]-, (C) [12]-, and (D) [13]-helicenes.

0036-8075/81/0918-1379$01.00/0 Copyright © 1981 AAAS

ied from 0.2 to 0.46 molal, with a nearly constant value (0.27 molal) for compounds **3** to **6** (Table 1). The mobile phase consisted throughout of a mixture of CH_2Cl_2 and n-C_6H_{14} (1:9), and the eluate was monitored at 254 nm (for other experimental conditions, see Table 1).

The data in Table 1 (see also Figs. 1 and 2) show chiral recognition on six of the seven columns examined, with resolution factors (r) varying from < 1.03 to 1.074. No resolution was observed for uridine (r = 1.00) under the experimental conditions, but no attempt was made to amplify any possible separation effect by recycling, as was done for 3′-AMP (Fig. 2). On a given column there was little difference between the r values for the various helicenes tested. The order of emergence on the adenosine derivatives was determined by simultaneous injection with optically pure helicenes (Table 1). In all such tests the $M(-)$ isomers were found to emerge first, and by extrapolation, this order is assumed to be generally valid on compounds **1** to **5**. This conclusion is supported by the constancy (though not necessarily identity) of the order of elution observed for helicenes on TAPA (*2*) and riboflavin (*1*).

The molecular mechanism for the chiral differentiation is not known because the necessary information on the structure of the pertinent diastereomeric helicene association compounds is not available. The solution to this rather complex problem requires a knowledge of the interaction of the helicenes with the respective nucleobases, the puckering of the sugar moiety and its relative orientation to the nucleobase, and the possible effect of the silica gel support. All of these structural features probably influence stereoselectivity. Ideas on the relationship of structure and chiral recognition, gained by a consideration of

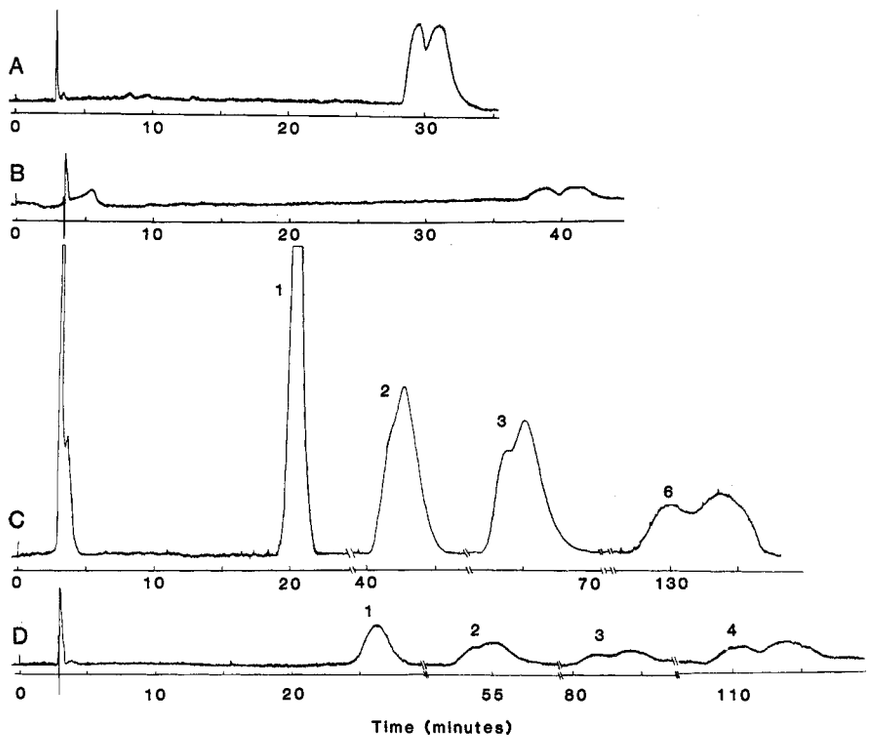


Fig. 2. The [12]- and [13]-helicenes on silica gel coated with 5′-AMP and 3′-AMP. Chromatographic conditions were as in Fig. 1, except that the column dimensions were 25 by 0.46 cm. The elution curves correspond to (A) [12]-helicene, (B) [13]-helicene on 5′-AMP, (C) [12]-helicene, and (D) [13]-helicene on 3′-AMP. The numbers above the peaks indicate the recycling steps.

Table 1. Chromatographic data for the enantiomeric helicenes on nucleosides and nucleotides coated on silica gel. The chromatographic conditions are given in Fig. 1; k_1' and k_2' correspond, respectively, to the isomers eluting first and last; the resolution factor $r = k_2'/k_1'$. The percentage of material coated is computed by weight.

Coated material	Amount coated (%)	Amount coated (mole/kg)		Capacity and resolution factors of helicenes				
				[14]	[13]	[12]	[11]	[10]
Adenosine*	5.3 ± 0.58	0.199	k_1'	5.26	4.53	3.68	2.88	2.04
			k_2'	5.5	4.76	3.88	3.04	2.13
			r	1.046	1.051	1.054	1.056	1.044
2′-Deoxyadenosine*	10.56 ± 1.10	0.426	k_1'		7.62	5.82	4.38	3.39
			k_2'		7.89	6.06	4.52	3.52
			r		1.035	1.041	1.032	1.038
3′-AMP	9.23 ± 0.84	0.265	k_1'		~ 8.2‡	~ 6.6‡		
			k_2'					
			r		< 1.03‡	< 1.03‡		
5′-AMP*	9.43 ± 0.26	0.271	k_1'	10.40	9.81	7.22	5.78	4.72
			k_2'	11.17	10.44	7.64	6.11	4.97
			r	1.074	1.064	1.058	1.057	1.053
3′,5′-Cyclic AMP†	~ 10§		k_1'	12.37	12.31	10.66		
			k_2'	12.89	12.71	11.0		
			r	1.042	1.032	1.032		
Guanosine	7.74 ± 1.17	0.273	k_1'	6.28	5.61			
			k_2'	6.44	5.78			
			r	1.030	1.030			
Uridine	11.25 ± 0.05	0.460	k_1'	6.36	5.71			
			k_2'					
			r	1.0	1.0			

*Simultaneously injected optically pure $M(-)$ [10]-, [12]-, and [13]-helicenes emerged first. †Simultaneously injected optically pure $M(-)$ [12]-helicene emerged first. ‡The capacity factor was determined from the retention time in the first cycle, whereas the r values were estimated from the retention times after the last recycling step. §Estimated from the amount added to the silica gel, rather than by elementary analysis as in all of the other cases.

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

References and Notes

1. Y. H. Kim, A. Tishbee, E. Gil-Av, *J. Am. Chem. Soc.* **102**, 5915 (1980).
2. F. Mikeš, G. Boshart, E. Gil-Av, *J. Chem. Soc. D* **1976**, 99 (1976).
3. A. Pullman and B. Pullman, in *Physico-chemical Mechanisms of Carcinogenesis* (proceedings of an international symposium held in Jerusalem, October 1968), E. D. Bergmann and B. Pullman, Eds. (Israel Academy of Sciences and Humanities, Jerusalem, 1969), p. 12.
4. Y. H. Kim and A. Tishbee, *J. Chromatogr.* **207**, 245 (1981).
5. For the sake of the present comparison it is immaterial if it should be found on recycling that uridine gives r values slightly higher than 1.000.
6. H. Weil-Malherbe, *Biochem. J.* **4**, 351 (1946).
7. M. Sundaralingam, in *Structure and Conformation of Nucleic Acids and Protein-Nucleic Acid Interactions*, M. Sundaralingam and S. T. Rao, Eds. (University Park Press, Baltimore, 1975), pp. 487–524.
8. Y. H. Kim, A. Tishbee, E. Gil-Av, *J. Chem. Soc. D* **1981**, 75 (1981).
9. R. I. Chang, A. W. Wood, W. Levin, H. D. Mah, D. R. Thakker, D. M. Jerina, A. H. Conney, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4280 (1979); T. Meehan and K. Straub, *Nature (London)* **277**, 410 (1979).

23 March 1981

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

0036-8075/81/0918-1381$01.00/0 Copyright © 1981 AAAS