

NOTES

Cyclopentanes and Petroleum Origin

BY BENJAMIN T. BROOKS

Cyclopentanes and cyclopentenes are not found in nature in plants or essential oils and five carbon ring structures are only very rarely found in complex substances such as pyrethrin, jasmone as a minor component in oil of peppermint and in the perfume oil of jasmine, chaulmoogric acid and combined with other ring structures as in a few of the triterpenes and isoprenic steroids. Cyclopentanes are found in the gasoline fractions of all types of petroleum, and in many cases, in substantial proportions. The careful analyses of seven petroleum made by Rossini¹ and his co-workers show the following percentages of cyclopentanes in the gasoline fraction distilling in the range 40–180°.

Crude source	Total cyclopentanes, per cent. by volume
Ponca, Okla.	8.86
East Texas	11.03
Bradford, Pa.	5.49
Greendale, Mich.	3.32
Winkler, Texas	5.46
Midway, Calif.	13.61
Conroe, Texas	5.66

In view of the extreme rarity of the five carbon ring structure in nature, the presence of cyclopentanes in petroleum is certainly not due to degradation of any natural products containing such structures. It is suggested that the cyclopentanes in petroleum have been formed by rearrangement of cyclohexanes. Such rearrangements have been observed experimentally at temperatures as low as 25° by the action of moist aluminum chloride.² It is believed that the occurrence of cyclopentanes in petroleum gives support to the theory that active surface minerals act catalytically in forming the wide diversity of hydrocarbons found in petroleum. Other evidence for this has been more fully presented in other papers.³

There is no constant relation between the per cent. of cyclohexane and methylcyclopentane and no relation between the ratios of these hydrocarbons and present bottom hole temperatures. The ratios of these two hydrocarbons do not correspond in the case of the gasolines noted, to the calculated thermal equilibrium compositions for any particular small temperature range and the high ratio of methylcyclopentane to cyclohexane found in some cases, 4.03% methylcyclopentane

to 2.04% cyclohexane in East Texas gasoline, is far out of line with the equilibrium compositions found experimentally (in the presence of aluminum chloride). At 77.4° the experimental equilibrium mixture contained 25.6% methylcyclopentane and lower percentages at lower temperatures. The lowest ratio of methylcyclopentane to cyclohexane in any of these gasolines was 2.97 to 4.34 in the Conroe, Texas, gasoline, or 39% of the sum of these two hydrocarbons. The composition of gasolines is full of similar inconsistencies when reference is made to calculated thermal equilibrium compositions. One such calculation led to a temperature of petroleum formation within the range of commercial cracking processes, a condition which is clearly precluded by the time element as well as many of the facts as to chemical composition of crude petroleum.

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Enzymatic Hydrolysis of Steroid Hormone Esters by Blood Serum¹

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The solubilities of testosterone, progesterone and estradiol in mammalian blood serum are accounted for by the solubilities of these substances in solutions of the serum constituents.^{2,3} Testosterone propionate and estradiol benzoate,⁴ are much less soluble in solutions of serum constituents than the corresponding free steroids, but the solubilities of these esters in serum on equilibration approach the solubilities of the free parent steroids. These results can be explained only by postulating the existence of a serum steroid hormone esterase.

Proof for this enzymatic hydrolysis by rabbit, bovine and human serum has been established using the Folin-Ciocalteu phenol reaction with estradiol benzoate as substrate. The benzoate ester gives no color with the phenol reagent, while estradiol develops the characteristic blue color, following Beer's law over a wide range. Serum or serum diluted with 6% bovine albumin is incubated with estradiol benzoate (0.2 mg. per cc.), which is added in ethanol so that final ethanol concentration is below 10%. Serum heated for one hour at 63° serves as a control for enzyme activity. Heated and unheated sera without substrate serve as controls for sera constituents reacting with the phenol reagent. The reaction is ended by precipitation at 80 to 90% ethanol concentration, removing the proteins which would react with the phenol reagent.

(1) A. F. Forziati, C. B. Willingham, B. J. Mair and F. D. Rossini, *Refiner*, Nov. (1943).(2) A. L. Glasebrook and W. G. Lovell, *THIS JOURNAL*, **61**, 1717 (1939).(3) B. T. Brooks, *Am. Assn. Petr. Geol.*, **32**, 2269 (1948); **33**, Sept. (1949).(1) Aided by a grant from the Donner Foundation, Incorporated.
(2) F. Bischoff and R. E. Katherman, *Am. J. Physiol.*, **152**, 189 (1948).(3) F. Bischoff and H. R. Pilhorn, *J. Biol. Chem.*, **174**, 663 (1948).(4) F. Bischoff, R. E. Katherman and J. J. Moran, *Abst. 115th Meeting Am. Chem. Soc.*, 1949, p. 6C.

Any liberated estradiol is recovered (90 = 5%) in the filtrate. Aliquots of the filtrates are evaporated to dryness *in vacuo* at room temperature, and extracted with chloroform or acetone, which further removes chromogenic substances. After evaporation the residue is ready for the Folin-Ciocalteu reaction. The reaction mixture is taken to pH 7.0 before ethanol precipitation in order to prevent appreciable hydrolysis of unchanged estradiol benzoate during processing.

The following are typical results of estradiol liberated for 1 cc. of serum at 37.5°. Rabbit serum, pH 7.0 in albumin buffer for thirty minutes: 0.50 mg. estradiol. Control heated rabbit serum: 0.015 mg. estradiol. Human serum, pH 7.2 in albumin buffer for six hours: 0.18 mg. estradiol. Control heated human serum: 0.03 mg. estradiol. Bull serum, pH 8.3 for thirty minutes: 0.070 mg. estradiol. Control heated bull serum: 0.008 mg. estradiol.

The degree of hydrolysis of solutions of estradiol benzoate in aqueous albumin or sodium lauryl sulfate in the pH range and concentration used in the enzymatic reaction is of the low order found for serum heated one hour at 63° or ten minutes at 70°. The marked hydrolytic effect of unheated serum therefore proves the existence of a steroid hormone esterase.

The enzymatic action has also been demonstrated in rat, chicken and pigeon blood, and on the substrates estradiol dipropionate and estrone acetate.

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A Hydrogen Transfer Reaction of the Butylenes and Butyl Alcohols in Sulfuric Acid

BY WILLIAM H. CALKINS AND T. D. STEWART

Isobutylene, 2-butene and the four butyl alcohols each react in 96% sulfuric acid at 0° to yield up to 20% isobutane. This reaction was observed by us during a study of the alkylation of isobutane by 2-butene.¹

We had occasion to introduce 2-butene alone, as a gas, into a flask containing stirred sulfuric acid and nitrogen gas at atmospheric pressure; the reactor was fitted with a mercury manometer. With a rate of introduction of 10 ml./min. the pressure rose immediately to 7-8 mm., remained nearly constant for ten to fifteen minutes, and then started to rise again. When the 2-butene flow was shut off within five minutes the pressure immediately fell to practically its original value; but if it was shut off during the later rise, the pressure fell only 2-3 mm. and then continued to rise for an hour or more without further 2-butene input. The same behavior, and to the same degree, was observed with the rate of input 27 ml./min. The volume of the reactor system was 600 ml.; the sulfuric acid varied from 25-100 ml. The eventual pressure rise for a charge of about 150 ml. of 2-butene was 20-30 mm., which, when corrected to room temperature, corresponded to a 17% yield of gas.

A preliminary examination of the gas formed, by Orsat combustion, indicated butane. This was confirmed by an analysis performed for us by the Shell Development Company, using their Consolidated Mass Spectrometer, which indicated 4% by volume of isobutane, 1% isopentane and traces of possible hexanes, in the nitrogen. Routine analyses were later made by an infrared spectrophotometer (National Technical Laboratories), calibrated at the wave lengths 8.48, 9.78 and 10.2 μ against pure samples of normal butane, isobutane and isopentane, and

following the procedure of Brattain, Rasmussen and Cravath.²

This isobutane evolution is related to the conversion of the four-carbon alkenes and alcohols to liquid saturated hydrocarbons under similar, but not identical, circumstances.³ Its uniqueness lies in the high percentage of isobutane, which may be a precursor to the hydrocarbons of higher molecular weight. The reaction is characterized by the presence of an induction period of a few minutes, in the case of 2-butene, and by the fact that rate of gas evolution from any given solution is approximately first order. However, both the rate and amount of gas formation vary somewhat with different amounts of sulfuric acid used. Table I details typical results.

TABLE I

YIELDS OF GAS EVOLVED FROM BUTYLENES AND BUTYL ALCOHOLS IN CONCENTRATED SULFURIC ACID AT 0°

Alkenes	Volume of acid, ml.	Moles of reactant	Pressure increase, mm.	Molal conversion, %
2-Butene	25	0.0059	27	16
2-Butene	50	.0072	37	16
2-Butene	100	.0063	26	13
Isobutylene	25	.0061	32	18
Alcohols				
<i>n</i> -Butyl	25	.0066	15	7.4
<i>t</i> -Butyl	25	.0074	22	10
<i>t</i> -Butyl	100	.0063	30	15
<i>s</i> -Butyl	25	.0033	18	19
<i>s</i> -Butyl	100	.0066	32	14
Isobutyl	25	.0065	48	25
Isobutyl	100	.0065	49	22

In the case of the alcohols, solution was effected slowly at 0° in a small amount of the acid outside the stirred reactor; this solution was then added rapidly to the excess of acid. Gas evolution was followed manometrically and analysis showed it to be almost entirely isobutane.

The induction periods in the case of the alcohols were striking. For *n*-butyl it was seventy-five minutes, for isobutyl thirty minutes, for *s*-butyl five minutes, and for *t*-butyl no induction period was observable. This corresponds to induction periods for 2-butene and isobutylene of a few minutes and none, respectively.

The relative rates of isobutane formation, based upon that from 2-butene as unity and using 25 ml. of sulfuric acid, were approximately as follows: 2-butene, 1; isobutylene, *t*-butyl alcohol and isobutyl alcohol, 0.5; *s*-butyl alcohol, 1.7; *n*-butyl alcohol, 0.2. The half-life of the 2-butene reaction was twenty-five to thirty minutes. It may be noted also that whereas in general the gas evolution from a given solution seemed to be a first-order reaction, that from the tertiary alcohol or isobutylene was distinctly not, the end reaction being very slow.

It is tentatively suggested that this apparent dismutation and rearrangement is based, not upon simple reactions of the original materials, but involves some polymeric material, perhaps through dealkylation; until polymer is formed no isobutane is evolved. The problem then con-

(2) Brattain, Rasmussen and Cravath, *J. Applied Phys.*, **14**, 418 (1943).

(3) Ormandy and Craven, *J. Soc. Chem. Ind.*, **47**, 317T (1928). The conversion of alkenes into alkanes during alkylation and polymerization, usually accompanied by rearrangement, has been observed, for instance by Ipatieff and Pines, *J. Org. Chem.*, **1**, 464 (1936), and McAllister, *et al.*, *ibid.*, **6**, 647 (1941). The polymerization studies have not been concerned with the low-boiling products; in alkylation an alkane-alkene transhydrogenation apparently occurs. In the present case a given alkene or alcohol is the sole initial reactant.

(1) Stewart and Calkins, *THIS JOURNAL*, **70**, 1006 (1948).