trogen, the results indicate approximately a 35-fold increase in purity. Further increases in yield and purity should be obtainable as more information becomes available about methods of coagulating the complex and of adsorbing and desorbing the antigen.

As contrasted with the usual physical-chemical methods for protein isolation, this procedure is simple and involves comparatively mild environments for the protein, thus reducing denaturation. In those cases where the procedure is applicable, the method is direct, it depends on a biological property of the protein, and it is highly specific. It should separate proteins with different biological properties but of similar physical-chemical properties, where the conventional techniques would fail. The process may prove useful for the isolation of tissue-specific proteins (including those in disease states), viruses, or toxins (*4*).

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Continuous Elemental Analysis of Organic Compounds in Gas-Chromatographic Effluents

Abstract. The compounds emerging from the gas-chromatographic column are quantitatively converted to a mixture of CO_2 and H_2. These gases are separated by means of an auxiliary column. The ratio of C and H atoms of each substance is deduced from the areas of carbon dioxide and hydrogen peaks.

A simple method for continuous analysis of the carbon and hydrogen content of volatile compounds previously separated by gas chromatography has been developed.

Substances emerging from a gas-liquid partition column are quantitatively oxidized on copper oxide to form carbon dioxide and water (*1*). The water is reduced to hydrogen by finely divided iron (*2*). Carbon dioxide and hydrogen are then separated by means of an auxiliary column, their concentration in effluent gases being determined by a thermoconductivity cell operated at room temperature.

Thus, two separate peaks, one for carbon dioxide and the other for hydrogen, are obtained for each compound, the ratio between areas under the curves depending on the elementary composition of the substance, independent of its weight.

In the case of hydrocarbons the empirical formula may be directly deduced from the ratio of H_2 and CO_2 peak areas, while with oxygenated compounds only the ratio between carbon and hydrogen atoms is obtained.

The gas chromatograph employed is a model B "Fractovap" of the Società Carlo Erba, Milan, Italy, equipped with stainless steel columns and connected to a model 62 electronic integrator.

The column of the apparatus is connected by means of a silicon rubber fitting to a coiled silica tube 60 cm long, having an internal diameter of 6 mm. This tube is filled in the first section with copper oxide and in the second one with reduced iron on inert support and is heated to 725° ± 10°C by means of an electronically controlled furnace.

Combustion gases are first passed through a 4-m auxiliary column packed with acetonylacetone on Celite C 22 to separate CO_2 and H_2, and then analyzed in a thermoconductivity cell. The conversion of a number of volatile organic compounds (containing C, H, and O) to a mixture of carbon dioxide and hydrogen is found to be quantitative throughout a wide range of experimental conditions (that is, carrier gas flow rate, reaction tube temperature, size of the injected sample, and so on).

Figure 1 shows three typical chromatograms obtained from benzene, cyclohexane, and ethyl ether.

The values for the carbon-hydrogen ratio in the empirical formula of analyzed substances, as deduced from CO_2 and H_2 peak areas, are summarized in Table 1; these values compare favorably with the experimentally derived ratios.

Table 1. Carbon-hydrogen ratios in organic compounds as derived from chromatogram peak areas. Standard deviation of listed values in column 4 is more than 3 percent. Benzene was used for calibration.

Substance	Peak areas (cm^2)		Ratio of peak areas (H_2 : CO_2)	Carbon-hydrogen ratio	
	H_2	CO_2		Calculated	Observed
Benzene	634	140	4.53	1.00	1.00
Cyclohexane	515	55.6	9.26	2.00	2.04
Ethyl ether	547	47.9	11.42	2.50	2.52

A small variation in the method's sensitivity—that is, in the ratio of peak areas to sample weight—was observed from day to day. When precise results are required, it is advisable to calibrate the apparatus by means of a standard substance having a known carbon-hydrogen ratio before running a series of analyses.

The proposed method, as compared to the conventional gas-chromatographic technique, offers the remarkable advantage of determining, by means of a thermal conductivity detector, a molecular parameter (such as the ratio of C and H atoms in the empirical formula) which allows, in most cases, direct identification of eluted compounds. Moreover, the detector sensitivity is markedly improved, as long as the katharometer can be operated at low temperature.

From the analytical point of view there is the advantage that separation and carbon-hydrogen ratio determination of such a minute amount as 0.05 mg of an organic substance contained in a complex mixture can be simultaneously performed.

Quantitative combustion, resulting in a mixture of carbon dioxide and hydrogen, of substances separated by gas

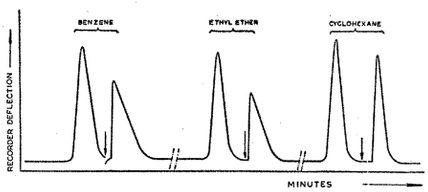

Fig. 1. Analysis of benzene, ethyl ether, and cyclohexane. Main column: 2 m long, internal diameter 6 mm, packed with a mixture of 25 parts by weight of di-*n*-decyl-phthalate on Celite C 22 (40 to 70 mesh). Temperature, 70°C. Auxiliary column: 4 m long, internal diameter 6 mm, packed with a mixture of 28 parts by weight of acetonylacetone on Celite C 22 (40 to 70 mesh). Temperature, 18°C. Carrier gas, nitrogen (flow rate, 0.7 lit./hr). Vertical arrows indicate polarity inversion of potentiometric recorder and sensitivity increase of the thermoconductivity detector. Ratio, 1:4 for benzene and 1:8 for cyclohexane and ethyl ether peaks.

chromatography may also be very useful when a continuous radiometric analysis of effluent gases is desired (*3*), as in the quantitative determination of both C^{14} and H^{3} in doubly labeled compounds (*4*).

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25 April 1960

Esters from Bacterial Oxidation of Olefins

Abstract. Identification of esters isolated from culture fluids of bacteria growing upon terminal olefins indicates that bacteria oxidize olefins at the saturated methyl group, leaving the double bond intact. The yeast *Candida lipolytica* produces α-glycols from olefins, presumably by attacking the double bond.

Utilization of straight chain olefins for energy and carbon sources among microorganisms is recognized (*1*), but few data are available to indicate the mechanism involved in the oxidation of these compounds. Bruyn (*2*) isolated *n*-hexadecanediol-1,2 from cultures of *Candida lipolytica* growing at the expense of hexadecene-1. Recent reports (*3–5*) have indicated that paraffins are oxidized by bacteria at the terminal carbon, probably via 1-alkyl hydroperoxide formation. Strains of Gram-negative coccoidal bacteria have been used in the laboratory of the department of bacteriology, State University of Iowa, to study the oxidation of alkanes containing an even number of carbon atoms, from C_{10} to C_{18} (*5*). The organisms grew well when a variety of olefins constituted the sole carbon source, and search was instituted for materials arising from bacterial oxidation of olefins in the liquid culture fluids.

Under conditions similar to those used in prior work (*4*), two strains of the hydrocarbon bacteria grew profusely in minerals–hexadecene-1 media, and a solid material of low melting point was isolated from each of the culture fluids. The material did not respond to any glycol tests but yielded a strongly positive hydroxamic acid test for esters. Growth and ester production were followed (Fig. 1); isolations were carried out at periods of peak ester formation, and the isolated material was subjected to mass spectrometric and infrared analysis (*4*). Mass analysis indicated the material to be an ester of molecular weight 478, with characteristic fragment ions:

$$m/q = 257 \quad (C_{15}H_{31}CO_2H_2^+)$$
$$= 256 \quad (C_{15}H_{31}CO_2H^+)$$
$$= 222 \quad (C_{16}H_{30}^+)$$

The infrared spectrum showed an ester carbonyl group and a sharp absorption peak at 11.0 μ in CCl_4 characteristic of $-CH{=}CH_2$. Thus, the data suggest an ester $C_{15}H_{31}CO_2CH_2(CH_2)_{13}CH{=}CH_2$ (15-hexadecenylpalmitate).

Analysis of material isolated from octadecene-1 cultures proved more difficult to evaluate and indicated the situation with respect to ester formation from the C_{18} olefin to be more complex. Mass spectra of such material showed ions of m/q = 285, 271, and 257, with relative intensities 1–2, 7–8, and 2–3, respectively, and of m/q = 250, with relative intensity 16. Since m/q=250 would correspond to $C_{18}H_{34}^+$ and m/q= 285, 271, and 257 would be $C_{17}H_{35}CO_2H_2^+$, $C_{16}H_{33}CO_2H_2^+$, and $C_{15}H_{31}CO_2H_2^+$, respectively, the mass spectra suggest the product to be a mixture of octadecenyl stearate, octadecenyl margarate, and octadecenyl palmitate in the ratios 1–2 to 7–8 to 2–3. It appears that bacteria attack 1-olefins at the saturated terminal carbon rather than at the double bond and, in the case of this organism, form esters in which the acid moiety is reduced (or totally synthesized via conventional fatty acid synthesizing pathways). These findings are in accord with those of Stewart and Kallio, who found esters (predominantly palmitate) formed from bacterial action on normal alkanes in the even-numbered series from C_{12} to C_{18} (*4*).

The data appear to contradict the observations of Bruyn (*2*), and a reassessment of the action of *Candida lipolytica* on hexadecene-1 was undertaken. After growth of *C. lipolytica* (ATCC 8661) for 5 days on minerals—hexadecene-1 media, ether extraction of culture fluids yielded a white crystalline material identical to that described by Bruyn. Infrared spectra of the isolated material and authentic hexadecanediol-1, 2 were indistinguishable. The isolated compound showed no carbonyl function in its spectrum and was negative to the hydroxamic acid test, but was cleaved by periodic acid. The physical constants of the material isolated were identical with those of authentic hexadecanediol-1,2. Total "diol," as determined by periodate titration, and C, H, and O content were also consistent with the conclusion that the material was essentially pure hexadecanediol-1,2.

It is thus possible to confirm the findings of Bruyn with respect to olefin oxidation by yeast. Evidently there are at least two pathways by which microorganisms initiate oxidation of terminal olefins: by direct attack at the double bond, or via oxidation of the methyl carbon at the saturated end of the molecule (*6*).

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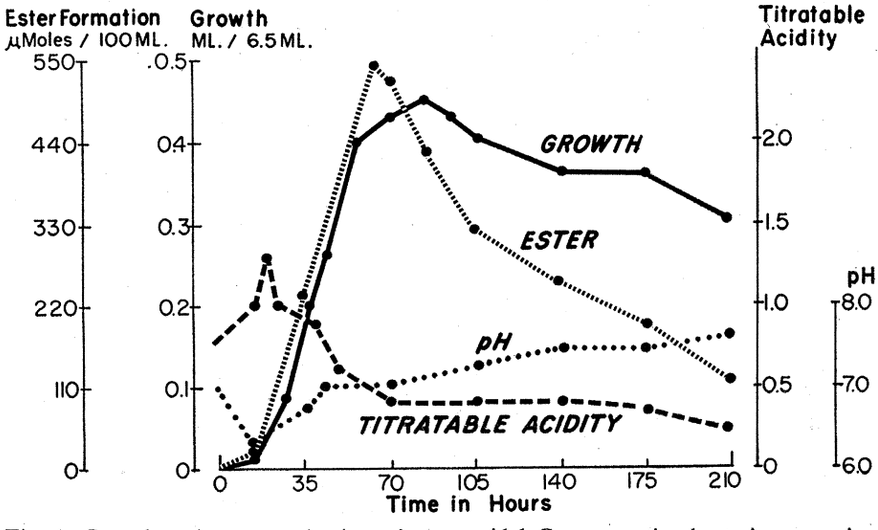

Fig. 1. Growth and ester production of a coccoidal Gram-negative bacterium growing aerobically in a minerals–hexadecene-1 medium. The ester was calculated as equivalents based on the ester group of cetyl palmitate (*4*).