that the only significant products of low molecular weight (*i. e.*, in the water-soluble oligosaccharide range) are maltose, amylotriose and amylotetraose. These findings suggest that the substrate molecules, having once diffused close enough to the enzyme molecule that enzyme–substrate complex formation can take place, are attacked by the enzyme with sufficient speed that the fragments initially produced are further attacked before they have time to diffuse away from the enzyme molecule. This hypothesis has now been tested by conducting enzyme digests under unfavorable conditions of *p*H or temperature and examining the products by means of paper chromatography. At intermediate stages of salivary amylase–amylodextrin digests we find a complete array of amyloöligosaccharides, covering the range from maltose (even small amounts of glucose) to amylooctaose and higher unresolved saccharides. Similarly with beta amylase–amyloheptaose digests, maltose and amylopentaose are the initial products of the reaction at 26° and *p*H 10, the amylopentaose accumulating and finally disappearing as amylotriose and maltose are produced in increasing amount. Similar effects were obtained at *p*H 4.7 and 70°.

We interpret these findings to mean that either the enzyme–substrate affinity constants are seriously decreased at higher temperatures or unfavorable *p*H, or the rate at which the enzyme–substrate complex disintegrates into products is diminished, or possibly a combination of these two effects. Further studies, in progress, may show the quantitative roles of affinity, disintegration and diffusion under conditions favorable and unfavorable for rapid enzyme action.

It is generally recognized that the optimal conditions of *p*H and temperature for a given enzyme may vary with different substrates or with variation in the kinds and amounts of salts, etc., present. However, in the present case, it has been demonstrated that not only the *rate* but also the *relative amounts of the intermediate products* of an enzymatic reaction are dependent on the reaction conditions.

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RECEIVED FEBRUARY 11, 1950

CHROMATOGRAPHY ON TREATED FILTER PAPER[1]

Sir:

In purifying the 2,4-dinitrophenylhydrazones of aldehydes and ketones by the chromatographic separation method of Roberts and Green,[2] combinations of derivatives were found which could not be separated. Because of the time involved in column chromatography, we used the test-tube paper chromatographic method of Rockland and Dunn[3a] as applied to 2,4-dinitrophenylhydrazones by Keller, *et al.*,[3b] and found that it can be adapted as a rapid checking system for the evaluation of numerous solvents in order to select one that can be used to separate these derivatives on corresponding chromatographic columns. The modification consisted in impregnating the paper with silicic acid so that the paper strip acts as a micro-silicic acid column.

Flood[4] has used impregnated papers for inorganic ion analysis in which the paper was first treated with aluminum hydroxide or a synthetic zeolite and then with a suitable reagent for developing the spot test. Hopf[5] has used Flood's method as a spot test for ketones and aldehydes by treating the filter paper with alumina and 2,4-dinitrophenylhydrazine. In the present instance the 2,4-dinitrophenylhydrazones were applied directly to the paper as explained by Keller, *et al.*[3]

Before cutting the filter paper in strips it was treated as follows: A heavy grade of filter paper was soaked in sodium silicate solution (Bé 42° diluted with two volumes of water), drained of surplus liquid, and then immersed for five minutes in 6 *N* hydrochloric acid solution, followed by a brief washing to remove excess acid. Care should be taken to avoid excess washing, which will leach out some of the silicic acid. The paper was then dried at 110° and finally pressed between weights to remove most of the curl in the treated paper.

Treatment of the paper in this manner broadens the scope of paper chromatography for application to more fields and compounds, and has been tried on other separations with excellent results. In some cases separations could be achieved which could not be obtained on any of the filter papers available on the market. For example as shown in Table I, the separation of three compounds can be achieved using treated paper where untreated paper fails. The method can be applied to any adsorbent such as starch, sugar or calcium hydroxide, which can be impregnated into filter paper.

TABLE I

COMPARISON OF R_f VALUES FOR 2,4-DINITROPHENYLHYDRAZONES ON TREATED AND UNTREATED FILTER PAPER

Solvent = 5% ethyl ether in petroleum ether (b. p. 110°)

Ketone	R_f Untreated paper	R_f Treated paper
Methyl ethyl	0.90	0.38
Methyl propyl	.90	.54
Methyl isopropyl	.91	.48

Where separation of compounds on packed chromatographic columns can be achieved in the time that it takes the solvent to travel the length of the column, duplicate chromatograms on

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[1] Report of a study made under the Research and Marketing Act of 1946.

[2] J. D. Roberts and Charlotte Green, *Anal. Chem.*, **18**, 335 (1946).

[3] (a) L. B. Rockland and M. S. Dunn, *Science*, **109**, 539 (1949); (b) G. J. Keller, J. G. Kirchner and R. G. Rice (unpublished).

[4] H. Flood, *Anal. Chem.*, **120**, 327 (1940).

[5] P. P. Hopf, *J. Chem. Soc.*, **785** (1946).

treated paper can be used to locate colorless bands on the columns.

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RECEIVED JANUARY 31, 1950

THE ISOLATION OF A CONJUGATED UNSATURATED ACID FROM THE OIL FROM *XIMENIA CAFFRA* KERNELS

Sir:

We have examined kernel oils from *Ximenia caffra Sond.*, *X. caffra var. natalensis Sond.* and *X. americana var. microphylla Welw.* The absorption spectra all showed a well defined maximum at *ca.* 230 mμ, and subsidiary maxima at *ca.* 268 and 280 mμ. The specific extinction coefficients at 229 mμ for the three oils were, respectively, 15.4, 12.6 and 12.0. The oils thus appeared to contain a considerable proportion of a conjugated dienoic acid and a smaller amount of a conjugated trienoic acid. Previous workers[1,2] have not reported the presence of such acids.

The acids from *X. caffra oil* were subjected to low-temperature crystallization. The fraction soluble in ether at −40° and insoluble in acetone at −60° was esterified and fractionally distilled. Fractions, b. p. 157–168° (0.1 mm.), with specific extinction greater than 40, were resaponified and the acids recrystallized from acetone at −15°, yielding a white solid m. p. 35°; four recrystallizations from petroleum ether yielded white plates, m. p. 40–41°; specific extinction at 229 mμ, 58.3; neut. equiv. 281.2; iodine value (Woburn, one hour[3]), 142.9. *Anal.* Calcd. for $C_{18}H_{30}O_2$: C, 77.65; H, 10.86; neut. equiv. 278.4. Found: C, 77.68; H, 10.81. The *p*-phenylphenacyl ester had m. p. 61.5–62°. *Anal.* Calcd. for $C_{32}H_{40}O_3$: C, 81.31; H, 8.53. Found: C, 81.31; H, 8.66. In a catalytic hydrogenation, 3.07 moles of hydrogen were absorbed, two moles immediately and the third during two to three hours. The product was stearic acid.

From these chemical data and the relatively low specific extinction at 229 mμ ($\Delta^{10,11,12,13}$-octadecadienoic acid has a maximum at 234 mμ and a specific extinction coefficient of 119[4]), it is postulated that this acid is a C_{18} acid with a double and a triple bond either in conjugation with one another or with one of them in conjugation with the carboxyl group. To locate these bonds the acid (1.7 g.) was oxidized with potassium permanganate.[5] There were isolated *n*-heptoic acid (0.5 g.) and azelaic acid (1.01 g.); and oxalic acid (0.18 g.) was determined volumetrically. The unknown acid, for which the name Ximenynic acid is proposed, could thus have one of the following structures:

$$CH_3(CH_2)_5CH{=}CHC{\equiv}C(CH_2)_7COOH$$
$$CH_3(CH_2)_5C{\equiv}CCH{=}CH(CH_2)_7COOH$$
$$CH_3(CH_2)_5CH{=}CH(CH_2)_7C{\equiv}CCOOH$$
$$CH_3(CH_2)_5C{\equiv}C(CH_2)_7CH{=}CHCOOH$$

From the spectral data, it is present in the oils in a concentration of *ca.* 25%. Full studies of ximenynic acid, and the ximenia oils will be recorded later.

We thank the South African Council for Scientific and Industrial Research for permission to publish these data, and Mr. F. Schoening for carrying out the elementary analyses.

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(1) S. V. Puntambekar and S. Krishna, *J. Indian Chem. Soc.*, **14**, 268 (1937).

(2) H. A. Boekenoogen, *Fette und Seifen*, **46**, 717 (1939).

(3) J. von Mikusch and C. Frazier, *Ind. Eng. Chem., Anal. Ed.*, **13**, 782 (1941).

(4) B. A. Brice, M. L. Swain, B. B. Schaeffer and W. C. Ault, *Oil and Soap*, **22**, 219 (1945).

(5) W. B. Brown and E. H. Farmer, *J. Chem. Soc.*, 761 (1935).

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RECEIVED JANUARY 19, 1950

NEW BOOKS

Boron Trifluoride and Its Derivatives. By HAROLD SIMMONS BOOTH, Hurlbut Professor of Chemistry, Western Reserve University, and DONALD RAY MARTIN, Assistant Professor of Chemistry, University of Illinois. John Wiley and Sons, Inc., New York, N. Y., 1949. ix + 315 pp. 40 figs. 14 × 22 cm. Price, $5.00.

The principles of electron-donor-acceptor bonding, which are necessary to the understanding of many catalytic processes and unpredictable aspects of valence theory, are so well illustrated by the chemistry of boron trifluoride that the publication of a complete summary of this subject is an event of major interest. The new book by Booth and Martin thoroughly covers the literature (to January, 1948) of compounds containing the B–F bond, including substitution derivatives, addition compounds and complex ions. The first three chapters describe the early history, preparation and manufacture, and physical properties of boron trifluoride, with an introductory summary of its chemical properties. The reader thus is prepared for the longer chapter on the coördinating power of boron trifluoride, the full presentation of the fluoborates and their substitution derivatives, and the very useful summary of the applications of boron trifluoride as a catalyst. The remaining chapters, on analytical methods and practical techniques, should assist research workers in learning to work with such materials; in fact one finds throughout the book many practical aspects which now become available through the close contact of the authors with the fluorine industry.

The book is valuable chiefly as a source of facts and