10. Two isomeric O-benzoates of acetoacetic ester were prepared and examined. It is suggested that the spatial structures of the esters must differ from those expressed in the usual type of stereomers.

11. The results in the progressive chemical retrogressions of enolates are interpreted by the primary formation of polymolecules with definite intermolecular structures. The reactions illustrate the general rule "that all substitutions, or pseudosubstitutions, proceed progressively, whether the several like or unlike replaced atoms or groups are attached to different carbons or to a single carbon." Thus, the synthesis of ketene and trimethylene by the action of zinc on bromoacetyl bromide and trimethylene bromide proceed through $CH_2(ZnBr)COBr$ and $CH_2(ZnBr)CH_2CH_2Br$, respectively, as intermediates with subsequent elimination of zinc bromide.

CAMBRIDGE, MASS. RECEIVED OCTOBER 19, 1935

[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY, SCHOOL OF HYGIENE AND PUBLIC HEALTH, THE JOHNS HOPKINS UNIVERSITY]

A Qualitative Test for Linolenic Acid, its Value and Limitations

BY GUSTAV J. MARTIN

A new reaction which affords a qualitative test for linolenic acid has been found in the behavior of this acid toward arsenophosphotungstic acid. The reagent, arsenophosphotungstic acid, is prepared as for the determination of uric acid by the method of Benedict.[1] The test is applied by layering 1 cc. of oil over 5 cc. of the reagent, heating for one hour in a boiling water-bath, and noting the color developed in the reagent layer. A positive reaction is indicated by the development of a deep blue color. The reaction will not take place in a medium such as acetone, alcohol or ether.

TABLE I

RESULTS OBTAINED WITH THE TEST WHEN APPLIED TO VARIOUS MATERIALS

Material	Reaction Qual.	Reaction Quant., %	Linolenic content, %
Methyl linolenate (prepared by method of Rollett[2])	+++++	Used as standard	99.8
Methyl linolate (prepared by method of Rollett[2])	....	......	0
Methyl linolate (crude)	+++	9 ± 3	?
Sodium oleate (Merck's U. S. P.)	....	......	0
Linseed oil	++++	81 ± 9	34[2]
Perilla oil	++++	36 ± 6	41[2]
Soya bean oil	+	3 ± 1	2.2[2]
Corn oil	....	......	0
Cotton seed oil	....	......	0

The reaction would probably be given by acids more highly unsaturated than linolenic acid, but as such acids do not occur in vegetable oils, the test is specific for linolenic acid or its esters in these oils. In applying the color test to fats or oils of animal origin, it may well be that arachidonic acid, and perhaps other highly unsaturated acids, would make the test non-specific.

The application of this color test in the determination of an unknown oil is easily recognized. There are only four vegetable oils considered in Jamieson's monograph[3] which would give an intense color with arsenophosphotungstic acid—linseed, perilla, chia seed and hemp seed oils. Another class, containing about 2% of linolenic acid would give a weak color test. Included in this group are such oils as soya bean, lumbang, mustard seed and rape seed oils. Only a total of about twenty vegetable oils would give a positive reaction and these could be subdivided as indicated. The test might be used to indicate adulteration or rancidity in the oils of the group, as linolenate content would primarily be affected in either case. It would only be necessary to compare the color developed by the oil in question to that developed by a known sample of the oil.

It is of interest to note that arsenic acid, phosphoric acid, sodium tungstate and phosphomolybdic acid do not give this reaction with linolenic acid.

Quantitative Value.—The reaction is to be regarded, at present, as being only roughly quantitative. The difficulty in obtaining adequate miscibility of oil and reagent preclude to a certain degree the application of the reaction to exact quantitative measurements. Using a purified methyl linolenate as a standard, a series of determinations was made on various materials as indi-

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(1) S. R. Benedict, *J. Biol. Chem.*, **92**, 161 (1931).

(2) E. T. Rollett, *Z. physiol. Chem.*, **62**, 410, 422 (1909).

(3) G. S. Jamieson, "Vegetable Fats and Oils," Chemical Catalog Co., New York, 1932.

cated in the table. The results indicate the average value obtained from a series of ten determinations. The variations in results demonstrate the inaccuracy of the method in its present form. Ten cubic centimeters of the reagent was used with the equivalent of 0.1 cc. of linolenic acid. The mixture was heated for one hour in a boiling water-bath. Comparison was made in a colorimeter with the standard.

Qualitative color tests for unsaturated fatty acids have not been reported previously. It is hoped that the introduction of this new type of reaction into fat analysis will lead to modifications of the tedious processes now necessary in qualitative analysis of fats and oils.

At the suggestion of G. S. Jamieson, of the United States Department of Agriculture, the above test was applied to oiticica and tung oils, which he supplied.[4] Tung oil contains elaeostearic acid, isomeric with linolenic acid, and oiticica oil contains a keto acid having three double bonds. The test was applied in a purely qualitative manner, with the result that the tung oil was found to give no reaction, while the oiticica reacted positively. The significance of this further observation cannot be interpreted at present, due to lack of knowledge of the structural formulas of the acids concerned. The suggestion was made by Dr. Jamieson, as he has demonstrated the presence in these oils of the three double bond fatty acids mentioned. Therefore, the conclusions are drawn more conservatively to state that the test is positive for linolenic acid and certain other three double bond fatty acids.

(4) This work was done following submission of the paper for publication, and is therefore not incorporated in the body of the paper.

BALTIMORE, MARYLAND RECEIVED JUNE 8, 1935

[CONTRIBUTION FROM THE BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE]

The Abundance Ratio of the Isotopes of Potassium in Mineral and Plant Sources

BY A. KEITH BREWER

Within the past few years differences in the physiological behavior of the isotope of hydrogen have been shown to exist. Further it has been shown that the abundance ratio is not necessarily constant, since H^2 is apparently concentrated in certain plants. In the case of other elements no physiological isotope effect has been established definitely although it has been suggested for oxygen and has been the subject of some investigation for potassium.

Stoklasa[1] has obtained evidence to support his idea that the radioactivity of potassium is responsible for its action in plant growth. Loring and Druce[2] found the atomic weight of potassium in potato vines to be 40.5 ± 0.1, the potassium chloride so extracted being more radioactive than the normal. In contrast Hönigschmid and Sachtleben[3] observed no definite variation in the atomic weight of potassium from hop flower ash and from potassium tartrate. Heller and Wagner[4] obtained the normal atomic weight for potassium extracted from the roots and leaves of potatoes and beets. Again Lowry[5] obtained 39.111 ± 0.013 and 39.091 ± 0.016 for the atomic weights from cotton ash and from wheat flour ash, respectively, and concluded that in these cases there was no isotope effect. Baxter and MacNevin[6] found no variation in the isotope composition of potassium assimilated by plants from an examination of wood ash. A definite isotope effect in plants for potassium is, therefore, still undetermined.

Potassium is now known to have three isotopes with mass numbers 39, 40 and 41.[7,8] Measurements of the abundance ratio of these isotopes are few. In the case of K^{39}/K^{41} Dempster[9] obtained a ratio of 18 while Bainbridge[10] finds the ratio to be near 12.5. Recently Bondy, Johannsen and Popper[11] obtained ratios varying between 13.9 and 20.7 with an average near 16. The first measurements made in this Laboratory showed the ratio to be about 13.9 ± 0.4[12] while recent improvements in the experimental technique have

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(1) J. Stoklasa, *Ernähr-Pflanze*, **30**, 299 (1934).

(2) H. F. Loring and J. G. F. Druce, *Chem. News*, **140**, 34 (1930).

(3) Hönigschmid and Sachtleben, *Z. anorg. allgem. Chem.*, **213**, 365 (1933).

(4) K. Heller and C. L. Wagner, *Z. anorg. allgem. Chem.*, **200**, 105 (1931); **206**, 152 (1932).

(5) H. H. Lowry, THIS JOURNAL, **52**, 4332 (1930).

(6) G. P. Baxter and W. M. MacNevin, *ibid.*, **55**, 3185 (1933).

(7) A. O. Nier, *Phys. Rev.*, **48**, 283 (1935).

(8) A. Keith Brewer, *ibid.*, **48**, 640 (1935).

(9) Dempster, *ibid.*, **18**, 415 (1921).

(10) K. T. Bainbridge, *J. Franklin Inst.*, **212**, 317 (1931).

(11) Bondy, Johannsen and Popper, *Z. Physik*, **95**, 46 (1935).

(12) Brewer and Kueck, *Phys. Rev.*, **46**, 894 (1934).