should be studied. Such an investigation requires containers which are not attacked by lime. In the present study, four points have been obtained (Samples 1 and 2, Expt. 3; Sample No. 1, Expt. 6; Sample No. 6, Expt. 1 in Table I) which have an excess of lime with respect to the arsenic acid in solution. These samples showed no detectable etching of the flasks nor any significant amount of silica in either solids or solutions. The points were purposely grouped in the range of solution concentrations found for them in order to be sure that the basic salt is not formed.

An examination of the molecular ratios of lime to arsenic oxide in the solutions (Table 1, column 5) shows that tricalcium arsenate is the only one that can dissolve in water at 35° without hydrolysis. Robinson[7] has reported solubilities for tricalcium and dicalcium arsenates at 25° and that solution of these compounds takes place without hydrolysis. Assuming that there is little difference in the system at 25 and 35° one must infer that Robinson's data on the solubility of dicalcium arsenate represent a false equilibrium. Such an explanation appears logical in view of the difficulties encountered in obtaining equilibrium in the system as experienced by the writers[2] and others.[3,4]

It is also of importance to note that all the compounds are stable in solutions more basic at 35 than at 90°.[2] This observation is in accord with some unpublished data which indicate that the compounds are fairly stable at room temperature in the presence of a lime solution but are changed rapidly to more basic compounds at temperatures in the neighborhood of 90°.

Summary

1. A study of the system $CaO-As_2O_5-H_2O$ at 35° has been carried out.

2. Three compounds have been identified: dicalcium arsenate, $CaHAsO_4$; pentacalcium arsenate, $Ca_5H_2(AsO_4)_4$; and tricalcium arsenate, $Ca_3(AsO_4)_2$. No basic calcium arsenate was found.

3. Importance of the data from theoretical and practical standpoints has been discussed.

GENEVA, N. Y. RECEIVED APRIL 22, 1937

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[7] Robinson, *J. Agr. Research*, **13**, 281 (1918).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

Purines in the Plant Kingdom: The Discovery of a New Purine in Tea[1]

BY TREAT B. JOHNSON[2]

The recent discoveries in the field of vitamins and hormones, and their vital importance to the normal development and control of life processes, have stimulated a renewed interest today in the search for new organic constructions occurring in nature. The refined methods of analysis and the newer techniques which have been developed have made possible the separation and identification of many new organic compounds occurring in microchemical quantities only, which hitherto were not conceived as playing any part in nature's processes. There is no doubt that the occurrence of many new organic combinations will be revealed to us in the future, for the study of natural products has received a new impetus as a result of the application of the improved methods of experimentation.

The known purines occurring in the plant kingdom are the three methylated derivatives of xanthine, namely: caffeine, $C_8H_{10}O_2N_4$; theobromine, $C_7H_8O_2N_4$; and theophylline, $C_7H_8O_2N_4$. These may be considered, according to our present knowledge, as end-products of purine metabolism in plants. In addition to these there have also been found adenine, $C_5H_5N_5$,[3] and guanine, $C_5H_5ON_5$, in the form of glucosides, adenylic acid and vernine or guanosine, respectively, and the two corresponding deaminized or secondary purines xanthine, $C_5H_4O_2N_4$, and hypoxanthine, $C_5H_5ON_4$. This second group of non-methylated purines may be considered as degradation products of plant nucleic acids. When introduced into the animal organism, the methyl-

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[1] Preliminary notice published in *Science*, Vol. 85, pp. 431 (1937).

[2] I desire to express here my thanks to the following men for their kind help and coöperation in securing data for preparing this paper for publication: William E. Ford, Professor of Mineralogy, and Curator of Mineralogical Collections, for his report on the crystallography of the new plant purine; Dr. James M. Sprague, Sterling Research Assistaint, for analytical assistance and aid in the synthesis of tetramethyluric acid, and Dr. Frank H. Stadola, National Tuberculosis Association Fellow, for microchemical analyses.

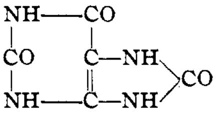
[3] Adenine-nucleotide from tea leaves, H. O. Calvery, *J. Biol. Chem.*, **68**, 593 (1926).

ated purines undergo a progressive demethylation, the order in which the methyl groups are removed varying in different species.[4] Whether demethylation is effected by oxidation or hydrolysis is not known. The exact function of purine alkaloids in plants is not understood. It is not known whether these purine constructions are metabolic intermediates or are products for excretion. Methylated uric acids have not been observed to date among the products of catabolism of the methylated xanthines in either the animal or plant kingdom. Evidence is accumulating that the metabolisms of purines in animals and plants have many points in common.

While uric acid has long been known to be a characteristic oxidation product in the animal catabolism of purines,[5] it is only within recent years that the occurrence of this oxypurine in plants has been demonstrated. It has been shown to occur in the spores of *aspergillus oryzae*.[6] Fosse[7] detected its presence in several plants but in very small quantities, about 30 to 250 mg. per kilogram of dry plant. According to this investigator this purine undergoes its normal and characteristic transformation in plant metabolism: one ferment changing it first into allantoin (plant uricase) and the other (allantoinase) into allantoic acid. Both of these two uric acid degradation products have been identified in plants.[8] *As far as the writer is aware, no methyl derivative of 2,6,8-trioxypurine I (uric acid) has thus far been shown to occur in nature.*

The author was fortunate in receiving recently for examination from Dr. F. E. Carruth,[9] a quantity of certain tea residues left after the commercial removal of the alkaloid caffeine from this plant. Dr. Carruth's description of the source of this material is as follows: "These residues were collected over the last few years and came from several million pounds of tea. They are customarily discarded in practice, but in this case a quantitiy was treated with methanol to wash out yellow vegetable coloring matters. After washing with methanol, the residue was taken into solution by warming with methanol. After filtering and cooling slowly, hexagonal crystals were noted mixed in with the other bases."

After an examination of this tea residue it was at once apparent to the author that it would be practical to separate the characteristic hexagonal crystals mechanically in sufficient quantity for chemical investigation. About 10 g. of the crystalline substance was picked out of the residue with small tweezers and thus easily obtained practically free from the other alkaloidal material present in the tea residue. This substance was purified without difficulty by crystallization from boiling water and was characterized by its crystal habit, its sharp melting point and neutral character. After a careful investigation of the several color tests applicable for the detection and identification of specific purine and pyrimidine constructions, the author has come to the conclusion that this new substance occurring in the tea residues furnished by Dr. Carruth is 1,3,7,9-tetramethyl-2,6,8-trioxypurine II. In fact, it is the first methylated derivative of 2,6,8-trioxypurine I (uric acid) to be discovered in nature.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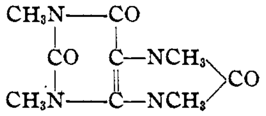
II

This purine derivative was first synthesized by Emil Fischer in 1884. Therefore, by the discovery of this purine II in tea, we not only increase the number of characteristic methylated purines occurring in the plant kingdom to four (theobromine, theophylline, caffeine and tetramethyluric acid), but we also stimulate a new interest in the possible natural occurrence of other methyl derivatives of this series, and also in the mechanism of the plant metabolism of purines in general.

The technique employed for identification of this new plant purine and the determination of its structure are described in the experimental part of this paper. A further study of the organic combinations occurring in tea extracts is now in progress.

Experimental Part

1,3,7,9-Tetramethyl-2,6,8-trioxypurine $C_9H_{12}O_3N_4$ from Tea Extracts.—It was possible to pick out with tweezers the glistening prisms of this purine from the crystalline tea mixture furnished to me by Dr. Carruth. These crystals were found to be very soluble in boiling water, and they separated on quickly cooling in the form of needle-

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[4] Kruger and Schmidt, *Ber.*, **32**, 2818 (1899); **32**, 2677 (1899); *Arch. exptl. Path. Pharmacol.*, **45**, 259 (1901); *Z. physiol. Chem.*, **34**, 549 (1902).

[5] Found in all animal urines, bird excrement, excretions of snakes and amphibians, insects, blood of mammals and in certain diseases.

[6] Sumi, *Biochem. Z.*, **195**, 161 (1928).

[7] Fosse, *Compt. rend.*, **194**, 1408 (1932); **195**, 1198 (1933).

[8] Fosse and Hieulle, *Compt. rend.*, **184**, 1596 (1927); Fosse, *ibid.*, **182**, 869 (1926); See also "Handbuch der pflanzen Analyse," G. Klein, 1933.

[9] Maywood Chemical Works, Maywood, New Jersey.

like prisms. On standing, however, in the laboratory at ordinary temperature these crystals redissolved, and were transformed during the crystallization into stout prismatic blocks. Individual blocks deposited on slow cooling which weighed 50 mg. Both the needle-like crystals and the stout blocks melted at the same temperature, 225°, to a clear oil without any decomposition or molecular rearrangement. The substance gave a good Murexide test and agreed, in all its properties, solubility, etc., with the description of 1,3,7,9-tetramethyl-2,6,8-trioxypurine given by Emil Fischer.[10] This purine can be purified by sublimation, and also be distilled under diminished pressure without decomposition. It is very soluble in hot water and boiling chloroform, moderately soluble in alcohol and difficultly soluble in ether.

Anal. Calcd. for $C_9H_{12}O_3N_4$: C, 48.21; H, 5.35, N, 25.00. Found: C, 48.03; H, 5.28; N, 24.96, 24.98.

Synthesis of 1,3,7,9-Tetramethyl-2,6,8-trioxypurine.—This purine was synthesized for comparison with the above compound separated from tea in the following manner. Anhydrous caffeine was first chlorinated in position 8 according to the directions of Fischer and Reese.[11] The resulting 8-chlorocaffeine was then allowed to interact with the required amount of sodium methylate in methyl alcohol solution when 8-methoxycaffeine was obtained in nearly a quantitative yield.[12] The latter purine was then rearranged according to Biltz's directions by heating above its melting point (200°), and was transformed smoothly into the required tetramethyluric acid.[13] This melted at 225–226° and when mixed with the compound isolated from tea extracts the melting point was not depressed. The purine crystallized from water, as described by Fischer, in either stout, heavy blocks or in long, fine needles. If one allows the needle-like crystals to stand suspended in their mother liquor for a long time, they gradually disappear and in their place are formed the heavy stout blocks. The substance which was separated from the tea residues exhibited the very same behavior when crystallized from water.

Crystallography of 1,3,7,9-Tetramethyl-2,6,8-trioxypurine from Tea

By WILLIAM E. FORD

The crystallographic and optical characters of Fischer's methylated purine (tetramethyluric acid) were described by Reuter.[14] The following is a summary of his results: monoclinic $a:b:c: = 1.789:1:1.914$; $B = 61°41'$. The forms present were a (100), c (001), R $(\bar{1}01)$, m (110), and x $(\bar{1}12)$. Two crystal habits were observed: (1) elongated parallel to c-axis and (2) even more elongated parallel to the b-axis. Perfect cleavages parallel to c (001) and m (110) optically. Indices in sodium light, $\alpha = 1.5384$, $B = 1.6093$, $Y = 1.7539$. Axial plane normal to (010) with $X = B$ axis and Z inclined 9.5° to C-axis in the acute angle B. $2V = 75°41'$. Dispersion horizontal, $r > x$.

The crystals obtained from the tea extracts have a brownish honey-yellow color and are nearly equidimensional in habit. They show the forms a (100), c (001), r $(\bar{1}01)$ and m (110). No faces corresponding to x $(\bar{1}12)$ were observed. The orthopinacoid faces were small but the other faces present were approximately of equal development. The habit is represented by Fig. 1.

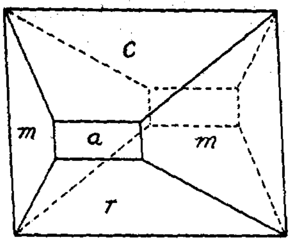

Fig. 1.

For the most part the crystal faces were curved and gave poor and multiple reflections of the goniometer signal, but in a few instances the crystal planes were of a better quality and from their measurement the following interfacial angles were obtained (column I) which are compared with the corresponding angles obtained by Reuter (column II).

	I	II
a (100) ∧ c (001)	61°43′	61°41′
c (001) ∧ r $(\bar{1}01)$	62°37′	62°27′
a (100) ∧ r $(10\bar{1})$	55°5′	55°44′
a (100) ∧ m (110)	57°32′	57°35′

The following observations confirm the optical description given by Reuter. Fragments of the crystals were immersed in oils of known refractive indices and studied under the microscope. The majority of the fragments lay on the basal cleavage. These for the most part showed an optic axis emerging just outside the field of vision, but in a few instances gave the axial figure well within the field. Practically all the fragments gave white of the higher order as the interference color and the axial figures showed many and closely crowded interference rings, thus confirming the unusual high birefringence. From the basal cleavage fragments the value of the index B was obtained as 1.60. The axial bar in the interference figure was only slightly curved, indicating a high value for $2V$. The optical sign

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(10) *Ber.*, **17**, 330, 1784 (1884); *ibid.*, **30**, 559, 3309 (1897); **32**, 2721 (1899); **28**, 2478 (1895).

(11) Fischer and Reese, *Ann.*, **221**, 336 (1883).

(12) Fischer, *Ber.*, **17**, 1785 (1884).

(13) Biltz and Strufe, *Ann.*, **413**, 200 (1917).

(14) A. Reuter, *Jahrb. Mineralogie*, **I**, 164 (1899); also *Ber.*, **30**, 3009 (1897).

was positive. Horizontal dispersion with $r > v$ was clearly observed.

Summary

1. The author has been successful in separating and identifying a new purine occurring in tea.

2. This was separated from concentrated tea residues after the commercial removal of caffeine.

3. The purine has been found to be identical with 1,3,7,9-tetramethyl-2,6,8-trioxypurine.

4. This is the first methylated derivative of 2,6,8-trioxypurine (uric acid) to be found in nature.

5. The study of plant extracts containing purines will be continued.

NEW HAVEN, CONN. RECEIVED APRIL 26, 1937

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

Ethylene. The Heat Capacity from 15°K. to the Boiling Point. The Heats of Fusion and Vaporization. The Vapor Pressure of the Liquid. The Entropy from Thermal Measurements Compared with the Entropy from Spectroscopic Data

BY CLARK J. EGAN AND J. D. KEMP

There has been some uncertainty previously, due to the possible persistence of rotation in the crystal below 10°K., as to whether the third law entropies of molecules containing symmetrically placed hydrogens would be the correct ones to use in thermodynamic calculations. This has been discussed recently,[1] with the conclusion "that very probably no molecules other than hydrogen and deuterium will have any residual entropy due to the persistence of rotation in the crystal at temperatures below 10°K." The results of the present measurements on ethylene, which contains symmetrically placed hydrogens, confirm the above conclusion.

Purification of Ethylene.—Ethylene, of 99.5% purity, from a cylinder, was passed through a tube containing phosphorus pentoxide and was condensed as a liquid in a previously evacuated bulb. The ethylene was cooled to a temperature slightly above the melting point and the gaseous impurities were removed by pumping with a mercury diffusion pump. The liquid was distilled in a vacuum-jacketed fractionating column under a pressure of 10 to 20 cm., the middle third portion being collected. The above procedure was repeated twice. The three middle portions were combined and fractionated again.

Due to an unusually rapid rise in the heat capacity curve below the melting point, it was at first thought that the ethylene contained a rather large amount of impurity. A further, more careful investigation of the heat capacity curve and of the extremely small variation of the melting point with the percentage melted disclosed that the rapid rise was actually the course of the true heat capacity curve. From the premelting effect shown by the heat capacity measurements very close to the melting point, it was estimated that the liquid-soluble, solid-insoluble impurity in the ethylene was less than 0.001 mole per cent.

Apparatus and Measurement of Amount.—The calorimetric apparatus and procedure have been described previously.[2] The calorimeter of laboratory designation Gold Calorimeter IV was used for the investigation. After the completion of the measurements, the amount of ethylene was determined using the 5-liter measuring bulb described by Giauque and Johnston.[3] The expression

$$\text{Amount in moles} = PV \times \frac{d}{M} \times \frac{1}{1 + \alpha t}[1 - \lambda(1 - P)]$$

was used in the calculation of the amount, where V = volume in liters, P = pressure in international atmospheres, d = density of the gas in grams per liter at standard conditions, M = molecular weight, α = mean coefficient of thermal expansion for the range 0 to 25°C., λ = coefficient of deviation from Boyle's law per atmosphere, and t = the temperature in °C. The pressure measurements were made using a Société Génévoise cathetometer as a comparison instrument in connection with a steel standard meter bar, and the pressure readings were converted to international atmospheres using the data in the "I. C. T." for the thermal expansion of mercury and of the meter bar. The corrections for capillary depression were taken from the work of Cawood and Patterson,[4] and the acceleration of gravity for this location was taken as 979.973 cm./sec.^{2}.[5] The standard ac-

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[1] Kemp and Pitzer, THIS JOURNAL, **59**, 276 (1937).

[2] Kemp and Giauque, *ibid.*, **59**, 79 (1937).

[3] Giauque and Johnston, *ibid.*, **51**, 2300 (1929).

[4] Cawood and Patterson, *Trans. Faraday Soc.*, **29**, 522 (1933).

[5] Sternwarte, Landolt, Börnstein and Roth, "Physikalisch-chemische Tabellen," Verlag Julius Springer, Berlin, 1923.