perature 20 to 35°. In performing some studies of conductance at high frequencies it was necessary to have solutions of accurately known dielectric constant. Dioxane–water mixtures have been used for this purpose, but our attempts to verify previously published data[2] for certain mixtures were not successful and so the entire system was redetermined. The data reported here enable use of dielectric constant to determine accurately the composition of dioxane–water mixtures.

Experimental

Materials.—The water used in these measurements was prepared by three distillations. Tap water was ordinarily distilled using a Barnstead still. The distillate was distilled in Pyrex equipment from an alkaline solution of permanganate. The middle third fraction from the second distillation was again distilled in Pyrex equipment retaining the middle third fraction which had a specific conductance of 1×10^{-6} ohms^{-1} cm.$^{-1}$.

Dioxane from the Carbide and Carbon Chemical Corporation was purified according to the method of Hess and Frahm[3] except the recrystallization was omitted. Time–temperature cooling curves were then run on the purified dioxane, employing an N.B.S. calibrated resistance thermometer and a Leeds and Northrup Co. Mueller Bridge. The freezing point was found to be 11.73° and the purity, as determined from the shape of the curves by the method of Rossini,[4] was not less than 99.95%.

Method.—For measurements of the dielectric constant of the various mixtures a General Radio Co. Twin-T Impedance Measuring Circuit was used.[5] A General Radio Co. Type 805-C Standard Signal Generator served as the frequency source. The detector unit consisted of a Hallicrafters SX-42 Receiver with a Du Mont Type 208-B Oscillograph for visual indication of the null point. All measurements were made at a frequency of one megacycle.

A new non-inductive type cell was constructed which is somewhat similar in design to one described by Ferry and Oncley,[6] and modified by Conner, Clarke and Smyth.[7] This cell is improved, as compared with previous ones of similar design, by the use of Teflon as the insulation material. Further, the lower Teflon plug and the inner electrode were threaded so as to permit complete dismantling of the cell. Several sizes of the central electrode were made to provide a range of capacitance. The metal parts of the cell were heavily nickel plated brass. At temperatures varying greatly from that of the room, temperature control within this water-jacketed cell may be not quite as precise as if the cell were in a thermostat, but this slight disadvantage is more than compensated for by the elimination of large lead corrections. Throughout all the measurements the temperature remained constant well within ±0.05°. The thermometer used was compared with an N.B.S. calibrated thermometer.

In the calibration of the cell, the dielectric constant of water was assumed to be 78.48 at 25° as reported by Albright.[8] All readings of capacitance from the Twin-T Circuit were corrected for drum variance using the manufacturer's calibration. The cell capacitance, $d\epsilon/dC$ was 1.83 $\mu\mu$f. and the lead capacitance was 1.4 $\mu\mu$f. To verify the method and to check the properties of the cell over a range of dielectric constant, the methanol-water system was studied at 25°. For this temperature the results of Albright and Gosting[9] were duplicated within one part in 1000.

Results

The values shown in Table I were obtained for the system dioxane–water at the temperatures shown.

TABLE I

DIELECTRIC CONSTANT FOR THE DIOXANE–WATER SYSTEM

Weight % dioxane	Temperature, °C. 20	25	30	35
0.00	80.38	78.48	76.72	74.97
10.00	72.02	70.33	68.74	67.10
20.00	63.50	61.86	60.38	58.96
30.00	54.81	53.28	51.91	50.60
40.00	45.96	44.54	43.33	42.24
50.00	36.89	35.85	34.81	33.88
60.00	28.09	27.21	26.45	25.74
70.00	19.73	19.07	18.58	18.07
80.00	12.19	11.86	11.58	11.26
90.00	6.23	6.07	5.96	5.85
95.00	3.99	3.89	3.83	3.76
100.00	2.24	2.21	2.20	2.19

Due to the low capacitance of the measuring cell the results in the low range of dielectric constant are limited to an accuracy of about one part in 200. The accuracy of the data at the higher dielectric constants listed is believed to be about one part in 2000. The value of the dielectric constant of pure dioxane at 25° is in excellent agreement with the value, 2.213, reported by Conner, Clarke and Smyth.[7] The value for pure dioxane differs from that given by Åkerlöf and Short[2] by one part in 20. In the low range our values for the mixtures differ from those of Åkerlöf and Short[2] by as much as 12%. The percentage difference decreases with increasing dielectric constant.

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The Introduction of Isotopic Hydrogen into Purine Ring Systems by Catalytic Exchange[1]

BY MAXWELL LEIGH EIDINOFF AND JOSEPH E. KNOLL

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Tritium or deuterium can be introduced by isotopic exchange in the presence of reduced platinum catalyst into atomic positions in adenine and guanine that do not exchange isotopic hydrogen under the usual conditions of aqueous acid or alkali. The presence of such non-labile atomic positions has been demonstrated in this Laboratory for adenine, guanine, cytosine and uracil using a biosynthetic procedure.[2] The catalytic exchange procedure described below makes it possible to prepare labeled products of tritium or deuterium content equal in order of magnitude to that of the aqueous medium used. The isotopic content of the products, relative to that of the reaction media,

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(2) M. L. Eidinoff, H. C. Reilly, J. E. Knoll and D. H. Marrian, *J. Biol. Chem.*, **199**, 511 (1952).

was used to calculate the extent of isotopic label incorporated (column 4, Table I). It is seen from the result of experiments 1–4 that catalytic isotopic exchange at 100° takes place in acidic medium or in an aqueous solution of the purine base. The recognized lability[3] of H in $-NH_2$ and $=NH$ leaves only the 8-position in guanine as the site of the isotopic label. The result in column 4 (expt. 5) indicates that the isotopic exchange did not go to completion under the experimental conditions. In adenine, the 2 and 8 positions may be sites for non-labile hydrogen. The isotopic content of the product is equivalent to slightly less than one equivalent atom position (method of calculation described below). The results (0.8–0.9) can signify either (a) substantially complete exchange at either the 2- or 8-positions or (b) partial exchange at both the 2- or 8-positions. Either possibility does not decrease the usefulness of the product for analyses using isotopic dilution techniques and for studies in which the adenine is not transformed into other compounds. Its usefulness in tracer metabolism studies depends on the location of the hydrogen isotope label. This method has been utilized to prepare adenine with tritium activity equal to 0.5 mc. per millimole using the conditions stated in experiment 3. The catalytic procedure described above will probably be useful for introducing isotopic hydrogen into other compounds having purine ring structures.

TABLE I

EXTENT OF ISOTOPIC EXCHANGE IN PREPARATION OF LABELLED ADENINE AND GUANINE[a]

Expt.	Compound	Medium	Equivalent no. of labeled atom positions
1	Adenine	70% AcOH + AcOT (no catalyst)	0
2	Adenine	70% AcOH + AcOT	0.8 ± 0.1
3	Adenine	H_2O + HTO	.85 ± .02
4	Adenine	D_2O	.91 ± .02
5	Guanine	H_2O + HTO 0.1 *N* HCl	.6 ± .1

[a] 18 hours at 100° for each run. Pt catalyst in all media except expt. no. 1.

Experimental

The platinum catalyst (250 mg.) was reduced in the reaction medium (Table I) with ordinary hydrogen gas at room temperature. The reaction mixture contained the reduced catalyst and 500 mg. of adenine or guanine in a total volume of 25 ml. After the contents had been frozen, the reaction tube was sealed off under vacuum. The tube was agitated at 100° for 18 hours. In experiment 4, the catalyst was reduced with 99.7% D_2 in 99.8% D_2O. The water used to prepare the media in experiments 1, 2, 3 and 5 had a tritium atom fraction of approximately 10^{-9}. The tritium was obtained from the Isotopes Division, U. S. Atomic Energy Commission.

The product in experiment 2, after heating with *N* sodium hydroxide for several minutes, was precipitated from hot solution as the hydrochloride. The products in experiments 3 and 4 were purified by recrystallization of the free base from ordinary water. The tritium content of the adenine was unchanged after standing in 0.01 *N* HCl solution for two days at room temperature and after boiling in *N* NaOH for five minutes. The product in experiment 5 was dissolved in hot *N* sodium hydroxide and precipitated as the sulfate. Deuterium or tritium bonded to nitrogen and oxygen would be replaced by ordinary hydrogen during the above purification steps. The purity of the products was checked by ultraviolet spectrophotometry (Beckman model DU instrument) using transmittance ratios at 250, 280 and 290 relative to 260 mμ. The infrared spectrum of the adenine prepared in experiment 1 was compared with that of a purified stock sample using a Model C Perkin–Elmer infrared spectrometer and rock-salt prism. The samples were prepared as a Nujol mull on a rock-salt window. The spectra were identical.

Isotopic Analyses.—The dried adenine and guanine samples were burned in a combustion train in which nitrogen oxides were reduced to nitrogen gas over copper at approximately 500°. The water was converted to hydrogen gas over zinc at 420°. Deuterium measurements were made using a dual collector Nier-type hydrogen mass spectrometer. Tritium measurements were made using a hydrogen–methane gas mixture in the proportional region as previously described by the authors.[4] The numerical values in column 4, Table I are equal to nf where n is the number of hydrogen atom positions undergoing exchange and f is the ratio of the D or T isotopic abundance in the n-positions to that in the aqueous exchange medium. For example, in experiment number 3, the tritium abundance in the medium was expressed as 9.6×10^5 counts per minute per standard volume of hydrogen gas, while the tritium abundance in the adenine prepared was 1.64×10^5 counts per minute per standard volume of hydrogen gas obtained from the combustion of the product. Taking the 5 hydrogen atoms into account $(9.6 \times 10^5)\, nf/5 = 1.64 \times 10^5$ and $nf = 0.85$. The result may be interpreted as showing the presence of one atom position undergoing catalytic exchange with $f = 0.85$ or possibly two exchangeable atom positions with average $f = 0.42$. Degradation studies would be required to resolve this question.

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Low Temperature Pyrolysis of Boron Trifluoride–Mannich Base Complexes. 2-Nitro-1-alkenes[1]

BY WILLIAM D. EMMONS, WILLIAM N. CANNON, JOHN W. DAWSON AND ROBERT M. ROSS

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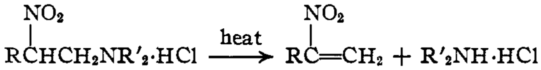
Recently Blomquist and Shelley[2] have demonstrated that Mannich base hydrochlorides derived from nitroalkanes undergo thermal decomposition[3] to yield the corresponding 2-nitro-1-alkenes. These investigators carried out the pyrolysis reaction at relatively high temperatures and this is certainly disadvantageous when heat-labile intermediates or unstable products are present in the reaction mixture. We have established that the use of boron trifluoride complexes of these Mannich bases effectively reduces the temperature necessary for carrying out the reaction by as much as 100°. This is in agreement with electronic theory which predicts that the carbon–nitrogen bond in this system will be weaker than in the corresponding hydrochloride. Furthermore the yields of nitroölefins obtained (80–90%) were as good as and in most



$$R\overset{NO_2}{C}HCH_2NR'_2\cdot HCl \xrightarrow{\text{heat}} R\overset{NO_2}{C}{=}CH_2 + R'_2NH\cdot HCl$$

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[1] This work was carried out under Army Ordnance Contract W-01-021-ORD-334.

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