into a mixture of DL-threonine and DL-allothreonine.[2] The N-formyl-O-methyl derivative that separated out from the first crystallization (m.p. 140–158°, sintering at 136°) was worked up yielding 0.58 g. of a white material, with a m.p. 213–216°. The final yield of recrystallized material was 0.443 g. (m.p. 232–234°) having an activity of 3.1 μc./mg. The material which was obtained in this manner on large scale runs[2] was reported to be essentially pure DL-threonine; however, under the present conditions, the material thus obtained assayed microbiologically only 53% of DL-threonine. On this scale it was further found that recrystallization of the water-insoluble N-formyl-O-methylthreonine isomer yields a product with a melting point of 164–170° (sintering at 147°) which, after hydrolysis and isolation as above, contains much less of a DL-allothreonine contamination. However, the over-all yield is reduced by 50% as a consequence of this second recrystallization.

DL-Allothreonine was isolated from the filtrate remaining after crystallization of the formyl derivatives according to the same technique as described above for DL-threonine. 0.294 g. of DL-allothreonine (m.p. 239–241°) was obtained. This material was combined with the filtrates from the threonine preparations, and worked up by the chromatographic method described below.

Chromatographic Separation of DL-Threonine and DL-Allothreonine.   Preparation of the Column.—A section of Pyrex tubing 16 mm. by 50 in. was constricted at one end in such a manner as to have a small bulb 1 cm. in diameter and an outlet tip 4 mm. in diameter. The bulb was loosely packed with glass wool and another 50-in. section of Pyrex tubing was attached to this by means of a short sleeve of rubber tubing. This column was mounted in position, temporarily closed at the bottom, and filled with water. A 6-in. funnel was attached to the top of the column and sufficient resin to fill the column was added as a slurry in 1.5 N HCl. This was permitted to stand until the resin settled to a constant level (40 hours) after which water was allowed to run through the column by gravity flow until the pH

of the effluent water was 6 (universal pH indicator paper).

The excess resin and water in the funnel and the upper 10 cm. of the column were removed with a pipet. The funnel and rubber tubing were detached and as soon as the resin stirred up by the above operation had settled, the water above the resin was removed.

Separation Procedure.—The C[14]-labeled DL-allothreonine and concentrated DL-threonine mother liquors as described above were dissolved in 6 ml. of water, forced into the column under 18 cm. pressure and followed with 5 ml. washings of water, each washing being forced into the column by pressure. Elution with 1.5 N HCl under 18 cm. pressure with the HCl reservoir level with the top of the column was then started. This gave an average flow rate of 5 ml. per hour.

After a forecut of 470 ml. had been collected, a fraction collector designed[11] to collect 15-min. samples was placed under the column and used for the remainder of the separation; 17- by 50-mm. shell vials were used as the receivers.

Analytical Procedure.—The location of the peaks of radioactivity was roughly determined by sampling and counting every tenth fraction. Then in the region containing the activity, 25-microliter aliquots were taken from each fraction and dried on polyethylene counting planchets, which were then counted for radioactivity. The results (Fig. 2)[12] indicate that a very nearly complete separation was effected.

Microbiological assays with *Streptococcus faecalis*, according to the method of Henderson and Snell,[13] indicated that the first compound eluted was DL-threonine and the second was the DL-allothreonine.

(11) Lien, Peterson and Greenberg, *Anal. Chem.* (in press).

(12) Since previous small scale separations of DL-threonine and DL-allothreonine gave regular, symmetrical peaks, the irregularities in the trailing edge of the allothreonine peak are not regarded as significant but are believed to be a result of either overloading of the column or counting irregularities resulting from an extremely high counting rate.

(13) Henderson and Snell, *J. Biol. Chem.*, **172**, 15 (1948).

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Exchange Reactions of γ-Pyrone and Synthesis of Deuterated Pyrones

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For spectroscopic purposes γ-pyrone, γ-pyrone-α-d_2, γ-pyrone-β-d_2 and γ-pyrone-d_4 have been prepared by synthesis and exchange. Exchange between γ-pyrone and deuterium oxide occurs readily at the α-position at pH 7. Lower pH retards the exchange and at higher pH γ-pyrone is unstable. The β-position shows no exchange under the same conditions. γ-Pyrone-d_4 was synthesized by the procedure of Claisen from the appropriate deuterated starting materials. γ-Pyrone-α-d_2 and γ-pyrone-β-d_2 were made by exchange from γ-pyrone and γ-pyrone-d_4, respectively.

γ-Pyrone and its three totally symmetrical deuterium derivatives γ-pyrone-α-d_2, γ-pyrone-β-d_2 and γ-pyrone-d_4 have been prepared to facilitate the analysis of the vibrational spectrum of γ-pyrone. For brevity, these compounds will on occasion be referred to as pyrone, α-d_2, β-d_2 and d_4, respectively. The infrared and Raman spectra and the spectroscopic interpretation will be published elsewhere.

Preparation of the d_4-compound was first attempted by exchange with deuterium oxide at pH 7, but only half the hydrogen atoms were found to exchange with appreciable rapidity. To determine whether these were the α- or β-hydrogens, exchange studies were then undertaken with 2,6-dimethylpyrone, in which the α-positions are blocked by methyl groups. It was presumed that the methyl hydrogens would not exchange under these conditions, and this presumption was verified. Furthermore the β-positions showed no ex-

change, from which the conclusion was drawn that in pyrone itself the α-positions are the active ones. Decrease in pH slowed down the rate of exchange.

These results are surprising, in that the usual exchange mechanisms would lead one to expect exchange in the β-position and increased rate of exchange at lower pH. It is possible that the mechanism is simply an ionic one in which the α-hydrogens are removed by direct attack by hydroxyl ion, followed by immediate replacement from the solvent molecules. The difference in rate of exchange between the α- and β-positions might then be due to greater positive charge on the α-carbon atom. Positive charge at the α-position would be expected from contribution to the electronic structure of pyrone by the resonance structure
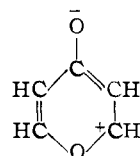
(1) Chemical Department, E. I. du Pont de Nemours and Co., Wilmington, Del.

whereas analogous structures with the positive carbon in the β-position cannot be written. Conceivably the inductive effect may also contribute significantly to the difference in positive charge at the two positions. The unusually large dipole moment of 2,6-dimethylpyrone (4.05 debyes observed vs. 1.75 calculated from bond moments[2]) indicates the presence of a center of considerable positive charge, and the α-carbon atoms are on these grounds the most likely location for it. Exchange studies on 3,5-dimethylpyrone and on electronically related molecules are planned in further confirmation of these results.

Since only the α-positions exchange, the α-d_2-compound can be prepared by this means, and β-d_2 also if the d_4-compound is available. The last-named was prepared in about the same way as pyrone itself, with appropriate deuterium-containing reagents. The method is given below.

Exchange Experiments and the Preparation of Pyrone-α-d_2.—Pyrone (0.1 mole) and 99.8% deuterium oxide (0.5 mole)[3] were heated together in a 25-ml. flask fitted with a reflux condenser and protected by a calcium chloride drying tube on an oil-bath at 95° for 18 hours. The pyrone was then separated from the solvent by vacuum distillation and its infrared spectrum taken. In addition to absorption in the C–H "stretching" region (3084 cm.$^{-1}$), absorption was also observed in the C–D "stretching" region (2289 cm.$^{-1}$), with the former somewhat more intense. The sample was then heated with another 0.5-mole portion of 99.8% deuterium oxide for an additional 18 hours. Had isotopic equilibrium been closely approached for all four hydrogen sites, the deuterium:hydrogen ratio in the pyrone should have been greater than 10:1. However, a Beer's law calculation based on the assumption that the extinction coefficient of the C–D band is half that of the C–H band indicated that the ratio of deuterium to hydrogen in the pyrone was 1.0. The result was verified by further heating of the partially deuterated pyrone with fresh deuterium oxide, which produced no further change in its infrared spectrum.

The observed exchange must have taken place preferentially either at the α- or β-position. In order to determine which position, 2,6-dimethyl-γ-pyrone (0.0024 mole) was heated with 99.8% deuterium oxide (0.25 mole) for 24 hours at 95°. The subsequent infrared spectrum of the dimethylpyrone indicated that no exchange whatever had occurred, either in the β-position or on the methyl groups.

Exchange studies of pyrone in deuterium oxide at pH 1 and 4 were also carried out. Although no quantitative rate determinations were made, the rate of exchange was definitely decreased by increasing acid concentration. Relative rates of exchange at the various acid concentrations were estimated by the ratios of the infrared optical densities at

the C–D and C–H frequencies of pyrone after a 24-hour exchange. It was impossible to observe the relative rates of exchange of pyrone with deuterium oxide in solutions of pH > 7 because of the high rate of hydrolysis of pyrone in alkaline solution.

Preparation of γ-Pyrone-d_4.—The completely deuterated compound was obtained by decarboxylation of chelidonic acid-d_4. The preparation of the chelidonic acid-d_4 was that described in reference 4 except for modifications necessitated by introduction of deuterium. The required acetone-d_6 (0.2 mole) was made by refluxing acetone with dilute solutions of sodium carbonate in 99.85% deuterium oxide, and the 120 ml. of concentrated deuterochloric acid needed for the ring closure step and for hydrolysis of ethyl chelidonate-d_2 was prepared by the method of Brown and Groot.[5] The crude ethyl chelidonate-d_2 was transferred to a 5-cm. buchner funnel, drained and washed with four 50-ml. portions of ordinary distilled water, cooled to 0°, to remove sodium chloride. The salt-free ester was dried in a vacuum desiccator over P_2O_5 for several hours. Yield was 47 g. (97%).

For hydrolysis, the dry ethyl chelidonate-d_2 was transferred to a one-liter, long-neck flask with calcium–chloride tube attached, 60 ml. of concentrated deuterochloric acid added and the mixture heated on the steam-bath for 30 hours. The yield of chelidonic acid-d_4, dried first for an hour under 20 mm. at 100° and then for one-half hour under 20 mm. at 160°, was 29 g. (79%). The removal of sodium chloride from the ester by washing was of prime importance, for when the hydrolysis was attempted without prior removal of the salt, the yield of chelidonic acid-d_4 was very small.

Pyrone-d_4 was obtained from chelidonic acid-d_4 by the method described by Willstätter and Pummerer.[6] Chelidonic acid-d_4 was intimately ground with about twice its weight of copper powder and dry distilled at 260–270°. Pyrone-d_4 distilled over and was collected at room temperature. The pyrone-d_4 was purified by vacuum distillation at 13 mm. and 97°. The yield was 5.6 g. (28%).

Preparation of Pyrone-β-d_2.—The β-d_2-compound was obtained by heating pyrone-d_4 with water. Pyrone-d_4 (0.037 mole or 0.074 equivalent of replaceable deuterium at the α-positions) was heated with water (1.0 equivalent of hydrogen) at 95° for 24 hours. This gave a calculated exchange of 93% of the deuterium atoms at the α-positions replaced by hydrogen. Because of the sensitivity of the pyrone ring to hydrolysis at elevated temperatures even in neutral solution, about 30% of the pyrone decomposes with each 24 hours of heating under these conditions. Therefore, only 2.2 g. of 93% pyrone-β-d_2 was obtained. Because of the small sample of β-d_2 and the large loss by hydrolysis on heating with water, it was felt inadvisable to try to improve the percentage of hydrogen at the α-positions beyond 93% by further exchange.

(2) E. C. E. Hunter and J. R. Partington, *J. Chem. Soc.*, **87** (1933).

(3) The deuterium oxide was obtained from the Stuart Oxygen Co., on allocation by the U. S. Atomic Energy Commission.

(4) E. R. Riegel and F. Zwilgmeyer, *Org. Syn.*, **27**, 40 (1947); L. Claisen, *Ber.*, **24**, 118 (1891).

(5) H. C. Brown and C. Groot, THIS JOURNAL, **64**, 2223 (1942).

(6) R. Willstätter and R. Pummerer, *Ber.*, **37**, 3733 (1904).