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## Preparation of 2-<sup>13</sup>C, 3-<sup>13</sup>C and <sup>15</sup>N Enriched Pyrroles. Conversion of Furan to Pyrrole

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In order to get further information on the molecular structure of pyrrole<sup>1</sup> by microwave investigation of isotopically substituted species we have prepared the necessary small quantities of 2-<sup>13</sup>C, 3-<sup>13</sup>C and <sup>15</sup>N enriched pyrroles. The microwave results will be published elsewhere. In a similar study on furan,<sup>2</sup> 2-<sup>13</sup>C and 3-<sup>13</sup>C enriched furans were synthesized, each in ca. 230 mg quantity, enrichment 22%. What remained of these samples (170 and 130 mg, respectively) was converted to the corresponding pyrroles by the action of gaseous NH<sub>3</sub> and a catalyst, but prior to these final experiments it was necessary to investigate the reaction between "ordinary" furan and NH<sub>3</sub> to ensure a reasonable yield of known purity. A 40–50% yield of this reaction has been reported recently<sup>3,4</sup> but this applies to the conversion of larger quantities. <sup>15</sup>N enriched pyrrole was prepared by pyrolysis of the ammonium salt of mucic acid, (CHOH)<sub>4</sub>(COO\*<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>, enriched to 33% in <sup>15</sup>N.

*Conversion of furan to pyrrole.* Initially, a dozen experiments each involving 500 mg furan were performed. Under comparable conditions (*vide infra*) the aluminium silicate cracking catalyst used by Yur'ev

(kindly given to us by A/G Deutsche Kalichemie) was found superior to  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> formerly used. The optimal temperature was fixed to 390°–410°C, a 50° rise or decrease in temperature causing a considerable smaller yield. Variation of the furan/NH<sub>3</sub> mole ratio from 1:5 to 1:8 indicated the advantage of working close to the latter limit which should not be exceeded because of the subsequent separation of reaction products. In literature, the magnitude of the optimal space velocity,  $v_s$ , defined as ml normalized gas volume per hour per ml catalyst, has never been given, although it is of great practical interest, it being widely independent of the scale of the experiment. We found that 100 <  $v_s$  < 200 gave satisfactory yields of 40–50% (*vide infra*).

In these 500 mg experiments the correct mixture of gaseous furan and NH<sub>3</sub> was fed at room temperature and atmosphere pressure to a U-shaped tube (i.d. 15 mm) containing 30 ml catalyst, the catalyst containing part of the U-tube being immersed in a heated metal-bath. The reaction products were condensed in a trap, cooled by dry ice-acetone. Removal of most of the excess of NH<sub>3</sub> took place *in vacuo* by pumping off controlled volumes of vapor fractions occurring at  $p = 300$ –100 mm Hg and  $t = -45^\circ$ –0°C. At 0°C, 5.00 ml of benzene was added to the remainder. The resulting solution of furan, pyrrole, H<sub>2</sub>O, NH<sub>3</sub> etc. in benzene was analyzed quantitatively on a gas chromatograph, (Perkin Elmer 116 E, column "R" [polypropyleneglycol], length 2 m). In this way any ambiguity in stating the true yield of the reaction, dating from a more or less wasteful separation of reaction products, was avoided.

In these 500 mg experiments the desired mixture of furan and NH<sub>3</sub> was obtained by mixing a needle-valve regulated stream of pure NH<sub>3</sub> (at room temperature and a pressure slightly higher than one atmosphere) of velocity 50 ml/min, with a stream of a mixture of furan and NH<sub>3</sub> (2:1) of the same temperature and pressure. This latter stream was fed to the NH<sub>3</sub>-stream by a mechanical pump which introduced Hg at a constant rate of 10 ml/min, displacing the furan/NH<sub>3</sub> mixture from a flask of 300 ml volume. In the final experiments involving 170 and 130 mg furan, respectively, all of the correct 1:7 furan/NH<sub>3</sub> mixture was contained in a 500 ml flask and introduced at a rate of 50–60 ml/min by the action of the pump.

It needs be added that the silicate catalyst had to be regenerated for removal of tar between runs in a stream of  $O_2$  at  $550^\circ C$ .

*Isolation of 2- $^{13}C$  and 3- $^{13}C$  enriched pyrroles.* The major part of  $NH_3$  was removed as described above. The  $0^\circ C$  remainder was distilled *in vacuo* into a tube, containing 10 g of BaO. After 1 h shaking the dehydrated product was again distilled *in vacuo* into a small tube. Here, vapor fractions were first removed at  $0^\circ C$ , later at room temperature ( $24^\circ C$ ), until the initially higher pressure had dropped to 7.5 mm Hg, the pressure of saturated pyrrole vapor. After a final distillation of the remanence, 34 and 25 mg enriched pyrrole, respectively, was collected, representing a 20 % yield with respect to furan. Infrared spectra of the vapors at room temperature ( $p = 7.5$  mm) in a gas cell of length 10 cm showed that these vapors contained no impurity (furan,  $NH_3$ ,  $H_2O$  etc.), detectable in the  $400-4000\text{ cm}^{-1}$  interval scanned on a Perkin Elmer Spectrophotometer 125 instrument. The spectra were, indeed, almost identical with the spectrum of ordinary pyrrole, the isotope effect being small. At the subsequent microwave investigation of the same vapors their purity was confirmed, and it could be seen that their isotopic enrichment is close to 22 %. Therefore, *no migration of  $^{13}C$  during the contact of the reaction mixture with the  $400^\circ C$  hot catalyst had taken place.*

*Preparation of  $^{15}N$  enriched pyrrole.* Since the furan to pyrrole conversion demands a considerable excess of  $NH_3$  it was found more profitable to prepare  $^{15}N$ -enriched pyrrole by pyrolysis of the ammonium salt of mucic acid,<sup>5</sup>  $(CHOH)_4(COOH)_2$ . 4.2 g (20 mmole) of mucic acid was stirred with 4 ml of water to a paste in a 60 ml flask and afterwards cooled and evacuated. 19.4 mmole gaseous,  $^{15}N$  enriched (33 %)  $NH_3$  was condensed and reacted with the acid in the same flask, after which excess water was removed *in vacuo* at room temperature. 6 g glycerol was added. After 12-24 h of standing the flask was connected to a special condenser for small quantities and kept for 15-20 min at about  $200^\circ C$ . The heterogeneous distillate (pyrrole,  $H_2O$ ,  $(NH_4)_2CO_3$ , tar, etc.) was afterwards distilled *in vacuo* at room temperature. The new distillate (about 1 ml) consisted of water and droplets of pyrrole. After two contacts with 2 g of CaO (stirring) water was removed, but CaO had liberated a small amount of  $NH_3$ , contaminating the pyrrole.  $NH_3$  was re-

moved and pyrrole isolated as described for the  $^{13}C$  enriched samples. The yield of  $^{15}N$  enriched pyrrole of satisfactory purity was 180 mg (270 mmole). This represents a 14 % yield with respect to the enriched  $NH_3$ , or about 4 times as much as obtainable by reacting 20 mmole of enriched  $NH_3$  to 3.00 mmole of furan.

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## A Method for the Introduction of Submicrogram Samples into a Gas Chromatograph

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In connection with quantitative analysis of steroids<sup>1</sup> the necessity arose of having at hand a simple method for the introduction of a known amount of a test mixture into the gas chromatograph. A number of well established methods of introducing liquids or solids into a gas chromatograph have been reported.<sup>2-4</sup> Such methods usually require modification of the injection system or rather elaborate devices.

The present paper describes a simple procedure for introduction of minute quantities into a gas chromatograph. The substance can be introduced with or without solvent. The absence of solvent has distinct advantages in quantitative analysis of steroids, fatty acids and amines, isolated from biological material. The major advantage is that a very minute sample can