THE SYNTHESIS OF PYRROLE DERIVATIVES LABELLED WITH ^{13}C IN SELECTED POSITIONS: IMPROVED PROCEDURES.

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SUMMARY

Starting from sodium acetate-1-^{13}C or -2-^{13}C improved procedures for the preparation of ethyl acetoacetate-2,4-$^{13}C_2$, ethyl acetoacetate-1,3-$^{13}C_2$, ethyl acetoacetate-2-^{13}C, and ethyl acetoacetate-2,3-$^{13}C_2$ are described. A method was also worked out which allows, using the labelled ethyl acetoacetates, to obtain Knorr's pyrrole (diethyl 3,5-dimethylpyrrole-2,4--dicarboxylate) carrying the label in well-defined positions in excellent yields. An example is given of further transformation of Knorr's pyrrole.

INTRODUCTION

In order to study the mechanism of α-side-chain substitution of 3,4,5-trisubstituted 2-methylpyrroles and the electronic effect of the substituents on the ipso position we needed pyrrole derivatives labelled with ^{13}C in selected positions. Since Knorr's pyrrole is known to be a very versatile intermediate for the preparation of a variety of pyrrolic compounds, we started an investigation aimed at finding out a method which permits selective labelling of its carbon atoms and at optimising reaction yields.

Diethyl 3,5-dimethylpyrrole-2,4-dicarboxylate is usually prepared from ethyl acetoacetate by the Knorr reaction[1].

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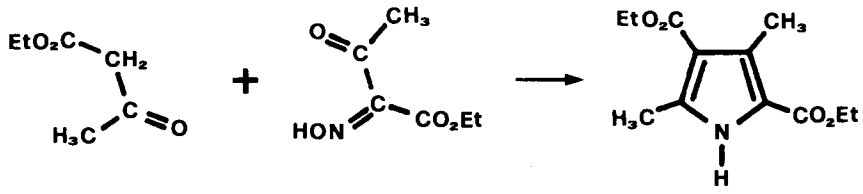
Apart from being not entirely satisfactory as far as its yield is concerned, this procedure is clearly not suitable to selective labelling since preparation is carried out in a single step. Therefore, we had to betake ourselves to another procedure[2] which involves preparation and isolation of ethyl 2-hydroxyimino--3-oxobutanoate and subsequent reaction with an equimolar quantity of ethyl acetoacetate in the presence of sodium amalgam as the reducing agent. This method is reported[2] to give better yields than the former one and seemed well suitable to our purpose provided ethyl acetoacetate labelled in appropriate positions could be conveniently obtained:



In the literature there are a few reports on the preparation of ethyl acetoacetate labelled with isotopic carbon. By the Claisen condensation reaction[3] ethyl acetoacetate-1,3-$^{*}C_2$[4], ethyl acetoacetate-2,4-$^{*}C_2$[5], and ethyl acetoacetate-1,2,3,4-$^{*}C_4$[6] have been prepared in low or only fair yields starting from ethyl acetate. Ethyl acetoacetate-1-$^{*}C$ has been prepared in very low yield from acetone and labelled diethyl carbonate[7] and in fair yield either by the Blaise reaction[8] or from acetyl chloride and benzyl ethyl malonate-3-$^{*}C$ in the presence of potassium tert--butoxide[8]. By the reaction of acetyl-1-$^{*}C$ chloride with sodium ethyl acetoacetate unsatisfactory yields of ethyl acetoacetate-3--$^{*}C$ have been obtained[9], whereas the reaction of acetyl-1-$^{*}C$ chloride with the ethoxymagnesium derivative of tert-butyl ethyl malonate[10] afforded the same compound in good yields[11].

Also ethyl acetoacetate-4-*C has been prepared by this method[12]. To our knowledge, neither ethyl acetoacetate-2-*C nor ethyl acetoacetate-2,3-$^{*}C_2$ have been prepared so far.

In this paper we describe improved procedures for the preparation of ethyl acetoacetate-1,3-$^{13}C_2$ (or -2,4-$^{13}C_2$), ethyl acetoacetate-2-^{13}C, and ethyl acetoacetate-2,3-$^{13}C_2$ starting from sodium acetate labelled with ^{13}C in either the 1- or in the 2-position, the synthesis of Knorr's pyrrole, and its conversion to ethyl 4-bromo-3,5-dimethyl-2-carboxylate. As usual, procedures were worked out and optimised using unlabelled materials.

RESULTS AND DISCUSSION

The Synthesis of Labelled Ethyl Acetoacetate.

For the synthesis of labelled ethyl acetoacetate we needed: ethyl acetate-1-^{13}C (ethyl acetoacetate-1,3-$^{13}C_2$), ethyl acetate-2-^{13}C (ethyl acetoacetate-2,4-$^{13}C_2$), t-butyl ethyl malonate-2-^{13}C or benzyl ethyl malonate-2-^{13}C (ethyl acetoacetate-2-^{13}C), acetyl-1-^{13}C chloride and either of the two mixed malonic esters (ethyl acetoacetate-2,3-$^{13}C_2$). Therefore, we had to search methods for the transformation of sodium acetate (commercially available either as $^{13}CH_2CO_2Na$ or $CH_3{}^{13}CO_2Na$) into the above-mentioned compounds.

Starting from sodium acetate, ethyl acetate has been prepared by the reaction with diethyl sulphate[13], triethyl phosphite[14], and ethyl bromide in DMF solution[15]. Although valuable as far as yield is concerned, all these methods suffer from the need of fractional distillations to purify the ester. To avoid this, we thought it useful to substitute HMPT for DMF in the last-mentioned procedure. In this way, a simple bulb-to-bulb distillation under reduced pressure sufficed to obtain a GLC-pure ester in nearly quantitative yield.

Mixed malonic acid esters may be prepared either from diethyl malonate by partial hydrolysis and subsequent re-esterification[10] or from an alkyl cyanoacetate by alcoholysis of the cyano group. The latter method was employed by Dahn and Hauth[8], who prepared benzyl ethyl malonate by ethanolysis of benzyl cyanoacetate. We found it convenient to adopt the same procedure since sodium cyanoacetate, which can be smoothly converted in very good yield into the benzyl ester by the method of Liu et al.[16], is an intermediate in the well-known reaction scheme utilised to prepare diethyl malonate-2-*C[17].

For the preparation of acetyl-1-^{13}C chloride, Dauben and Bradlow's procedure[11] proved to be entirely satisfactory.

In a subsequent step, either ethyl acetate or benzyl ethyl malonate had to be converted into ethyl acetoacetate. As to the latter, this was achieved in a straightforward manner by a combination of Hauser's[10] and Dahn's[8] methods (see Experimental). As to the former, none of the literature methods gave completely satisfactory yields, even when strong, weakly nucleophilic bases were employed, due to the occurrence of a number of side reactions. Therefore, we tried to modify the existing procedures in order to minimize these side reactions. As base, potassium hydride, which seems to have never been used for β-keto ester formation, appeared to be the reagent of choice due to its reactivity and the other advantages it has over the commonly used sodium hydride[18]. As solvents, ethers (especially THF and the glyme solvents) are recommended for use in reactions with KH[18]; THF, however, proved to be unadequate for the acetoacetic ester condensation reaction, probably due to the appreciable solubility of the metallated intermediates, which favours polyacylation of the ethyl acetate. The use of cyclohexane, wherein the potassium derivatives are nearly insoluble, prevented the side reactions from occurring to a considerable extent and,

consequently, very high yields in ethyl acetoacetate (85-93% with respect to ethyl acetate) - the highest ever reported - could be obtained.

The Synthesis of Labelled Knorr's Pyrrole.

As already pointed out, the only way to achieve labelling in well-defined positions of Knorr's pyrrole is first to prepare ethyl 2-hydroxyiminoacetoacetate and then to condense it with ethyl acetoacetate in the presence of a suitable reducing agent. By an appropriate choice of the position of the label either in the hydroxyimino derivative or in the acetoacetic ester or in both, Knorr's pyrrole labelled in any desired nuclear or nuclear and side-chain positions may thus be obtained.

Ethyl 2-hydroxyiminoacetoacetate may be prepared either in aqueous acetic acid[19] or in aqueous sulphuric acid[20]. Both methods afford approximately the same yield, which is only moderate and not very reproducible. In our hands, the subsequent reaction of the hydroxyimino derivative with ethyl acetoacetate in the presence of sodium amalgam[2], which is rather laborious, failed to give the desired product in yields better than 50%. Recently, Clezy et al.[21] described a two-step Knorr synthesis for the preparation of ethyl 3-methyl-4-oxo--4,5,6,7-tetrahydroindole-2-carboxylate in ca. 50% yield. Since their procedure, is by far less complex than the above-mentioned one, we tried to apply it to the preparation of Knorr's pyrrole which we obtained in excellent yields and with such a purity degree to make its recrystallisation unnecessary.

As an example of further transformation, Knorr's pyrrole was then converted into 5-ethoxycarbonyl-2,4-dimethyl-3-carboxylic acid, which, after protodecarboxylation, was brominated to quantitatively yield ethyl 4-bromo-3,5-dimethylpyrrole-2--carboxylate.

EXPERIMENTAL

Melting points and boiling points were not corrected. Thin-layer chromatographic analyses were performed on Merck F_{254} silica plates, benzene being the eluant, unless otherwise indicated. Gas-liquid chromatographic analyses were carried out on a C.Erba Fractovap mod. G.I. apparatus equipped with a FID detector and a Leeds and Northrup recorder. The column (length = 1 m, i.d. = 3 mm) was filled with 5% Carbowax 1500 on Chromosorb W. The carrier gas was nitrogen. Correct labelling was checked step by step by ^{13}C-NMR spectroscopy (solvent: $CDCl_3$, internal standard: TMS) using a Varian CFT-20 instrument.

MATERIALS

Sodium acetate-1-^{13}C and sodium acetate-2-^{13}C (anhydrous, 90% ^{13}C) were obtained from Sorin. The other reagents were reagent-grade products; they were purified as usual whenever necessary. Solvents were purified and dried according to the literature[22].

PREPARATIONS

Ethyl Acetate-1-^{13}C and Ethyl Acetate-2-^{13}. Sodium acetate--1-^{13}C (or -2-^{13}C) (2.0 g, 24.1 mmol) was suspended in anhydrous HMPT (16 ml), ethyl bromide (2.6 g, 24.1 mmol) was added and the mixture stirred overnight at room temperature. The ethyl acetate formed was isolated by a bulb-to-bulb distillation under reduced pressure (water aspirator, bath temperature: 60°C). The receiver (protected from moisture) was cooled with a liquid nitrogen bath. The yield of GLC-pure ethyl acetate-1-^{13}C (or -2-^{13}C) was always nearly quantitative (98-99%).

Acetyl-1-^{13}C Chloride. This was prepared in 90-93% yield starting from sodium acetate-1-^{13}C according to Dauben and Bradlow[11].

Ethyl Acetoacetate-1,3-$^{13}C_2$ and Ethyl Acetoacetate-2,4-$^{13}C_2$. The preparation was carried out under an argon atmosphere. In a three-necked flask equipped with a reflux condenser, a pressure-equalising dropping funnel, and a magnetic stirrer (the whole apparatus was weighed before beginning the experiment) was placed an appropriate volume of a homogeneous 50% suspension of potassium hydride in mineral oil (Fluka). The hydride was thoroughly washed three times with 20 ml portions of dry benzene and, finally, with 20 ml of dry cyclohexane. The washings were decanted each time into a toluene-isopropanol (1:1) mixture. Before re-weighing the apparatus plus hydride, any residual solvent was removed by heating under vacuum. To the hydride (ca. 1 g, 25 mmol), suspended in ca. 25 ml dry cyclohexane and heated at 50°C, was slowly added a solution of 2 equivalents (ca. 50 mmol) of ethyl acetate-1-^{13}C (or -2-^{13}C) in ca. 15 ml of dry cyclohexane under vigorous stirring. The additions were effected over a 1-h period during which temperature was gradually raised to 80°C. The mixture was then refluxed for 3 h. After cooling, the flask was connected to a distillation apparatus and the solvent removed first at atmospheric pressure (the oil-bath temperature was not allowed to exceed 120°C) and then in vacuo. The residue was dissolved in the minimum amount of ice-cold 50% aqueous acetic acid and the slightly acid solution extracted repeatedly (8-10 times) with 15 ml portions of diethyl ether. The combined extracts were washed with aqueous $NaHCO_3$ and with water, and dried over anhydrous Na_2SO_4. The ether was stripped off at room temperature under reduced pressure and the residue, a slightly coloured oil, was purified by distillation under reduced pressure. In order to avoid severe losses by volatilisation, the receiver was cooled in a dry ice-acetone bath. Yields of pure (GLC, TLC) ethyl acetoacetate (bp = 80-82°C at 30 Torr) varied between 83 and 92%.

Benzyl Ethyl Malonate-2-^{13}C. Sodium acetate-2-^{13}C (2.0 g, 24 mmol) was nearly quantitatively converted into bromoacetic--2-^{13}C acid according to Kögl et al.[23]. This was dissolved in 10 ml of water and sodium carbonate added to bring the pH of the solution to 8. Then the solution was cooled slightly, and KCN (2.2 g, 34 mmol) was added portionwise at such a rate to prevent the temperature of the reaction mixture to exceed 90°C. After the exothermic reaction had subsided, the solution was warmed at 95-100°C for 1 h. The solution was then brought to dryness in vacuo and the sodium cyanoacetate-2-^{13}C thus obtained was converted, without purification, into benzyl cyanoacetate-2-^{13}C by the phenyl dichlorophosphate method of Liu et al.[16]. The crude ester was purified by distillation in vacuo (bp = 108-110°C at 0.1 Torr) and transformed into benzyl ethyl malonate-2-^{13}C according to Dahn and Hauth[8]. The overall yield was 3.5 g (53%).

Ethyl Acetoacetate-2-^{13}C. To magnesium diethoxide[10] (3.6 g, 32 mmol) suspended in dry diethyl ether (30 ml) benzyl ethyl malonate-2-^{13}C (7.0 g, 32 mmol) was added with stirring. After a 30-min reflux, to the mixture a solution of acetyl chloride (2.50 g, 32 mmol) in the same solvent (10 ml) was added at such a rate as to maintain the ether gently refluxing. Refluxing was then continued for an additional 30 min. The cooled mixture was then treated with ice-cold water (10 ml) and slightly acidified with dilute sulphuric acid. The ether layer was removed and the aqueous phase extracted twice with Et_2O. The combined ether extracts were washed with water and dried (anhydrous Na_2SO_4). The solvent was stripped off under reduced pressure at room temperature, and the residue, a yellowish oil, was converted into ethyl acetoacetate-2-^{13}C by Dahn and Hauth's procedure[8]. The overall yield was 2.4 g (58%).

CONVERSION OF DIETHYL 3-METHYL-^{13}C-5-METHYLPYRROLE-2-^{13}C-2,4--DICARBOXYLATE INTO ETHYL 4-BROMO-3-METHYL-^{13}C-5-METHYLPYRROLE--2-^{13}C-2-CARBOXYLATE.

Ethyl 3-methyl-^{13}C-5-methylpyrrole-2-^{13}C-2-carboxylate. 12 mmol of diethyl 3-methyl-^{13}C-5-methylpyrrole-2-^{13}C-2,4--dicarboxylate was converted in 88% yield into 2-ethoxycarbonyl--3-methyl-^{13}C-5-methylpyrrole-2-^{13}C-4-carboxylic acid[24]. Since thermal decarboxylation[25] presents some drawbacks (the acid sublimes in part, yields are neither very reproducible nor particularly good), we preferred to use the following procedure.

The acid (10 mmol) was dissolved in 95% ethanol, 37% HCl (7.5 ml) was added, and the mixture gently refluxed for ca. 4 h. After cooling, the hydrochloric acid was carefully neutralised with aqueous Na_2CO_3 (saturated), the ethanol removed under reduced pressure, and the residue extracted with CH_2Cl_2. The dried (anhydrous Na_2SO_4) extracts were brought to dryness in vacuo and the residue, a light-brown solid, was purified by chromatography on silica gel (25 g, Merck, reinst), a mixture of ethyl acetate and benzene (1:1) being the eluant. Mp = 122-123 °C (lit.[25]: mp = 122-123 °C); yield: 97.5%.

Ethyl 4-bromo-3-methyl-^{13}C-5-methylpyrrole-2-^{13}C-2--carboxylate. The preceding pyrrole (11 mmol) dissolved in CCl_4 (11 ml) was brominated by slowly adding a solution of bromine (11 mmol) in the same solvent (6 ml) at room temperature. When the addition was complete, the mixture was stirred for an additional 15 min and any volatile material then removed under reduced pressure. The residue was taken up in a little petroleum ether (bp = 30-50 °C), filtered, and purified by recrystallisation from aqueous ethanol. Mp = 148-149 °C (lit.[26]: mp = 150 °C); yield 92%.

Ethyl Acetoacetate-2,3-$^{13}C_2$. The ethoxymagnesium derivative of benzyl ethyl malonate-2-^{13}C (16 mmol) was condensed with acetyl-1-^{13}C chloride (1.3 g, 16 mmol) and transformed into ethyl acetoacetate-2,3-$^{13}C_2$ as described above. The overall yield was 1.2 g (56%).

^{13}C-Labelled Knorr's Pyrrole. By condensing ethyl acetoacetate and ethyl 2-hydroxyiminoacetoacetate bearing the appropriate labels, Knorr's pyrrole, labelled either in one or more nuclear positions or in nuclear and side-chain positions, may be obtained. The procedure used was as follows.

Ethyl acetoacetate (10 mmol) in glacial acetic acid (7 ml) was converted into the 2-hydroxyimino derivative by adding sodium nitrite (0.71 g) in water (3.5 ml) at $T<15$ °C and leaving the mixture to stand at room temperature for 5 h. In a three-necked flask equipped with thermometer, reflux condenser, dropping funnel, and magnetic stirrer ethyl acetoacetate (10 mmol), aqueous acetic acid (90%, 14 ml), and anhydrous sodium acetate (2.6 g) were placed. The solution was warmed to 90 °C and zinc powder (2.3 g) was added at once with stirring followed immediately by the solution of the ethyl 2-hydroxyiminoacetoacetate, which was added at such a rate as to maintain the temperature of the reaction mixture between 95 and 105 °C. When the addition was complete, the mixture was heated to 105 °C for 15 min and then decanted, while hot, from the excess zinc into ca. 200 ml ice-cold water. The excess zinc was repeatedly washed with hot AcOH until the washings were colourless. After standing overnight, the snow-white product was collected, thoroughly washed with water, and dried in vacuo at 50 °C. Mp = 133-135 °C (lit.[1]: mp = 135-136 °C). Yields were in the range 80-88%.

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