

Perkin Elmer Spectrophotometer, Model 21. Microanalyses have been performed by Dr. A. Bernhardt, Mülheim.

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On an Isomerization in the Reaction of Aromatic Grignard Reagents with Alkyl *p*-Toluenesulphonates

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Alkyl sulphonates have been used as alkylating agents for aromatic compounds, either by thermal decomposition of the ester in the aromatic compound as solvent,¹ or by the reaction with an aromatic Grignard reagent². In the first mentioned type of reaction the substitution follows the general rules of electrophilic substitution although the selectivity appears to be lower than in Friedel-Crafts alkylations, as is evident by the large extent of *meta* substitution¹. The Grignard reagents on the other hand often give low yields due to side-reactions which may lead to alkyl bromides or reduction of the Grignard reagent². In any case it appears to be generally assumed that, in the alkylation, substitution occurs at the C-Mg bond². However, in attempts to prepare 2-isopropylthiophene from

2-thiophenemagnesium bromide and isopropyl *p*-toluenesulphonate we observed an isomerization and obtained mixtures of the isomeric isopropylthiophenes containing about 55 % of the 2-isomer and 45 % of the 3-isomer. The analyses were carried out using NMR-spectroscopy and measuring the methyl group bands of the two isomers. On metalation with *n*-butyllithium, followed by carbonation, the isomeric mixture gave the same proportions of 2-isopropyl-5-thiophenecarboxylic acid and 3-isopropyl-5-thiophenecarboxylic acid, which were identified by the characteristic coupling constants of disubstituted thiophenes³. This verifies the original analysis of the isopropylthiophene mixture. The reaction of 3-thiophenemagnesium bromide with isopropyl *p*-toluenesulphonates also proceeds with isomerization leading to a mixture of about 50 % of 3-isopropylthiophene and 50 % of 2-isopropylthiophene. Heating isopropyl *p*-toluenesulphonate in thiophene as solvent did not lead to alkylation.

Isomeric mixtures are also obtained in the reaction of 2-thiophenemagnesium bromide with ethyl *p*-toluenesulphonate and propyl *p*-toluenesulphonate (70 % 2-isomer and 30 % 3-isomer). No rearrangement of the alkyl chain in the latter case was observed.

It has also been found that, in contrast to earlier statements⁴, a mixture of *t*-butylthiophenes is obtained in the reaction of 2-thiophenemagnesium bromide and *t*-butyl bromide⁵.

Preliminary experiments indicate that the observed isomerization is not limited to the thiophene series. Reaction of *p*-methoxyphenylmagnesium bromide with isopropyl *p*-toluenesulphonate gives a mixture of isomeric isopropylanisols. We are studying the scope and mechanisms of these rearrangements, which in some way may be analogous to the rearrangements occurring in the reaction of certain aromatic zinc, mercury and cadmium compounds with acid chlorides⁶⁻⁸, which most probably proceed by a cyclic mechanism⁹.

It is clear that the isomeric purity of many compounds prepared by the reaction of unsymmetrical aromatic Grignard reagents with *p*-toluenesulphonates can be seriously doubted.

Experimental. 2- and 3-Isopropylthiophene. The Grignard reagent was prepared in the usual way from 48.9 g (0.3 mole) 2-bromothio-

phene. After refluxing for 20 min, 65.2 g (0.3 mole) of isopropyl *p*-toluenesulphonate in 300 ml dry ether were added at such a rate as to maintain gentle reflux. When the addition was complete the reaction mixture was refluxed for 2 h and then hydrolyzed by iced acid. The ether layer, after washing with water, was concentrated and then steam-distilled. The distillate was extracted with ether and the ether phase dried and distilled yielding 7 g (18 %) of a mixture containing 55 % 2-isopropylthiophene (A) and 45 % 3-isopropylthiophene (B) b.p. 151–156°, $n_D^{20} = 1.5054$. (Found: C 65.99; H 7.87; S 25.23. Calc. for $C_9H_{10}S$ (126.2) C 66.61; H 7.99; S 25.41). NMR-data: (CCl_4) τ_{CH_3} (A) = 8.71, $J_{CH_3-CH} = 7.0$ c/s τ_{CH_3} (B) = 8.79, $J_{CH_3-CH} = 7.0$ c/s

2- and 3-Isopropyl-5-thiophenecarboxylic acid. 50 ml of 1.1 N butyllithium were added to 7 g (0.055 mole) of isopropylthiophenes in 60 ml dry ether. After refluxing for 30 min the solution was poured on to dry carbon dioxide and 100 ml dry ether. The mixture was hydrolyzed with water and the water phase was acidified with dilute hydrochloric acid and extracted with ether. After removal of the ether 7 g (75 %) of the acids were obtained consisting of 54 % of 2-isopropyl-5-thiophenecarboxylic acid (C) and 46 % of 3-isopropyl-5-thiophenecarboxylic acid (D)

NMR-data: (acetone) τ_{H_3} (C) = 3.10, τ_{H_4} (C) = 2.37, J_{34} (C) = 3.7 c/s, τ_{H_3} (D) = 2.60, τ_{H_4} (D) = 2.27, J_{24} (D) = 1.7 c/s.

The NMR-spectra were recorded on an Varian Associates A-60 high resolution spectrometer.

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Preparation of ^{35}S -Labelled 6-Aminopenicillanic Acid

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In order to carry out certain pharmacological work with new penicillins prepared by reaction of 6-aminopenicillanic acid with different acid chlorides, it was desirable to have such compounds radioactively labelled in the heterocyclic moiety. From the technical as well as the economic point of view it seemed preferable to start with ^{35}S -labelled benzylpenicillin, which could readily be split into radioactive 6-aminopenicillanic acid and phenylacetic acid by means of enzymatic hydrolysis¹⁻⁴.

The biosynthetic preparation of ^{35}S -labelled benzylpenicillin has been previously described by Smith and Hockenull⁵ and by Perret⁶. They reported that 10–20 % of the ^{35}S added (as $Na_2^{35}SO_4$) was incorporated in the penicillin formed during the fermentation. In our experiments about 27 % of the radioactive sulphur added was present in the penicillin formed in the broth. About 60 % of this material could be isolated as crystalline potassium penicillin.

Experimental

Biosynthesis and isolation of ^{35}S -labelled benzylpenicillin.

Organism. *Penicillium chrysogenum* (Astra strain 1155), was grown on a sporulating medium of the following composition: 30 g crushed barley, 5 ml asparagine solution (0.1 % asparagine + 3 % glycerol in water) in a 250 ml conical flask.

Seed. The spores from one flask were suspended in 100 ml of sterile water. 2 ml of that suspension were used for seeding one 500 ml conical flask containing 100 ml of the following medium: Corn steep liquor 7.6 %, sucrose 2.0 %, $CaCO_3$ 1.0 %, and soy bean oil 0.25 %. pH was 6.2. A suitable number of flasks seeded in that way were incubated at 27°C for 72 h on a rotary shaker (220 r.p.m., two inches circular stroke).