NOTES.

280. *Reduction of αβ-Unsaturated Ketones with Lithium Aluminium Hydride and Aluminium Chloride.*

By J. Broome, B. R. Brown, A. Roberts, and A. M. S. White.

The literature [1] indicates that reduction of αβ-unsaturated ketones or alcohols with lithium aluminium hydride and aluminium chloride is not straightforward. We have confirmed that this reagent reduces the carbonyl group of an αβ-unsaturated ketone to a methylene group, but that behaviour of the double bond is uncertain, perhaps depending on the structure of the ketone. Similar behaviour, usually involving a double-bond shift, has been reported for reduction of αβ-unsaturated ketones by the Wolff–Kishner method.[2]

The most straightforward and useful reduction is that of ketones in which the double bond and the keto-group are in the same ring. Cholest-4-en-3-one gives a good yield of cholest-4-ene in one step, or when reduction is first with lithium aluminium hydride to the epimeric alcohols [3] and then to cholestene on addition of aluminium chloride. When cholest-4-ene-3,6-dione is formed into a complex with aluminium chloride in ether and then added to lithium aluminium hydride, the 3-keto-group is preferentially removed with the formation of cholest-4-en-6-one. Steroidal 4,6-dien-3-ones yield mixtures of dienes, as they do on Wolff–Kishner reduction.[2b]

If the double bond and the keto-group are not in the same ring, reduction may be more complex. For example, benzylideneacetone yields a mixture of but-1′-enyl- and butylbenzene, and the ultraviolet spectrum of the product from 1-acetylcyclohexene indicates that an appreciable amount of a diene is present.

The suggested route for the reduction of αβ-unsaturated ketones with this reagent is shown in the annexed scheme. Lutz and Gillespie [4] suggested that the reduction of an αβ-unsaturated ketone to a saturated alcohol with lithium aluminium hydride occurs through a cyclic intermediate. This is sterically impossible when the double bond and the keto-group are homoannular. Thus the reaction paths through the saturated alcohol (I)

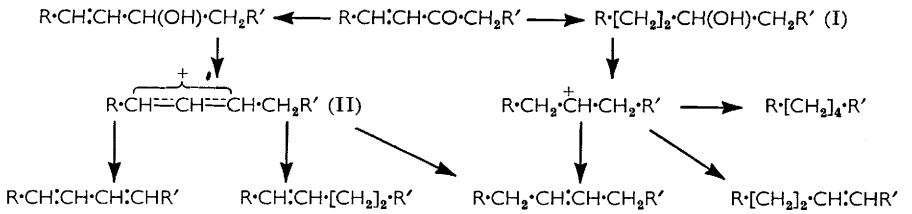
[1] Brown, *J.*, 1952, 2756; Broome and Brown, *Chem. and Ind.*, 1956, 1307; Birch and Slaytor, *ibid.*, p. 1524; Wheeler and Mateos, *ibid.*, 1957, 395; Albrecht and Tamm, *Helv. Chim. Acta*, 1957, **40**, 2216.

[2] (*a*) Lardelli and Jeger, *Helv. Chim. Acta*, 1949, **32**, 1817; Fischer, Lardelli, and Jeger, *ibid.*, 1950, **33**, 1335; (*b*) Eck, Van Peurse, and Hollingsworth, *J. Amer. Chem. Soc.*, 1939, **61**, 171.

[3] McKennis and Gaffney, *J. Biol. Chem.*, 1948, **175**, 217.

[4] Lutz and Gillespie, *J. Amer. Chem. Soc.*, 1950, **72**, 2002.

cannot occur. The behaviour of the carbonium ion (II) then controls the composition of the final product.



Experimental.—The general method of reduction was that previously described,[5] lithium aluminium hydride (1·75 mol.), aluminium chloride (3·5 mol.), and the ketone (1·0 mol.) in ether being used. Alumina was Spence's Grade 0. Light petroleum refers to the fraction of b. p. 30—40° unless otherwise stated.

Reduction of cholest-4-en-3-one. (*a*) Cholestenone (5·26 g.) gave cholest-4-ene, needles (from acetone) (4·19 g.), m. p. and mixed m. p. 80—81°, $[\alpha]_D^{18}$ +67° (in $CHCl_3$) (Found: C, 87·8; H, 12·0. Calc. for $C_{27}H_{46}$: C, 87·6; H, 12·4%). The dibromide was obtained as needles, m. p. and mixed m. p. 116—117°.

This experiment has been carried out successfully on a scale of 20—30 g.

(*b*) Cholestenone (2·60 g.) in ether (50 ml.) was run into a slurry of lithium aluminium hydride (2·50 g.) in ether (75 ml.). The mixture was boiled for 15 min. and then cooled in ice while a solution of aluminium chloride (2·80 g.) in ether was added. After the mixture had been boiled for 30 min., cholest-4-ene (2·23 g.), m. p. and mixed m. p. 80—81°, was isolated in the usual way.

Reduction of cholest-4-ene-3,6-dione. A solution of cholest-4-ene-3,6-dione (1·01 g.) and aluminium chloride (1·50 g.) in ether (50 ml.) was added to a slurry of lithium aluminium hydride (1·00 g.) in ether (50 ml.), and the mixture was boiled for 1 hr. By the usual method of isolation, followed by chromatography on alumina and elution with benzene, cholest-4-en-6-one (0·43 g.) was obtained. Crystallisation from acetone gave colourless needles, m. p. 106—107° (Found: C, 84·2; H, 11·1. Calc. for $C_{27}H_{44}O$: C, 84·3; H, 11·5%), $\lambda_{max.}$ 242 mμ (ε 6550 in EtOH). The infrared spectrum in Nujol had a strong band at 1686 $cm.^{-1}$. Reich, Walker, and Collins [6] record m. p. 108—109°, $\lambda_{max.}$ 243 mμ (ε 6400 in EtOH).

Reduction of cholesta-4,6-dien-3-one. Cholestadienone (0·60 g.) gave a crystalline product (0·55 g.) which was passed through alumina (25 g.) in light petroleum. Elution with this solvent (60 ml.) gave a solid which separated from acetone as colourless needles, m. p. 86—87·5° with previous softening. After two further crystallisations from alcohol, the product had m. p. 85—86°, $[\alpha]_D^{19}$ −39° (*c* 0·8 in $CHCl_3$), $\lambda_{max.}$ (in hexane) 229 and 236 mμ (ε 15,200 and 16,000), $\lambda_{infl.}$ 244 mμ (ε 10,500). A similar mixture, m. p. 82·5—84°, $[\alpha]_D^{26}$ −38°, was obtained [2b] by Wolff–Kishner reduction of the dienone.

Reduction of ergosta-4,6,22-trien-3-one. This ketone (1·20 g.) gave a slightly yellow solid. Chromatography in light petroleum on alumina gave the fractions: (1) (0·35 g.), m. p. 89—90° and $\lambda_{max.}$ (in EtOH) 230, 236, and 245 mμ (ε 26,600, 27,400, and 17,800); (2) (0·55 g.), m. p. 82—87°; and (3) (0·15 g.), m. p. 96—98°. Crystallisation of fraction (1) from acetone changed the m. p. to 83—89°. Fraction (2) separated from acetone as colourless plates of a mixture of 3,5,22- and 4,6,22-trienes, m. p. 83—84°, $[\alpha]_D^{20}$ −86° (*c* 2·5 in $CHCl_3$) (Found: C, 88·25; H, 11·8. $C_{28}H_{46}$ requires C, 88·4; H, 11·6%). Crystallisation of fraction (3) from acetone gave *ergosta-3,5,22-triene* as elongated plates, m. p. 99—100°, $[\alpha]_D^{19}$ −130° (*c* 4·6) in $CHCl_3$) (Found: C, 88·2; H, 11·3%), $\lambda_{max.}$ (in EtOH) 227, 234, and 243 mμ (ε 24,400, 25,300, and 16,800).

Reduction of 1-acetylcyclohexene. Acetylcyclohexene (10·5 g.) gave a pale yellow oil [9·3 g.; $\lambda_{max.}$ (in EtOH) 230 mμ (ε 3020)]. Vapour-phase chromatography showed the presence of two compounds (33 and 67%). The oil in light petroleum was passed through alumina (120 g.). Elution with light petroleum (400 ml.), evaporation, and distillation gave a colourless oil (5·50 g.), b. p. 135—136°/755 mm., n_D^{21} 1·4620 (Found: C, 87·3; H, 12·6. Calc. for C_8H_{14}: C, 87·3; H, 12·7%). The ultraviolet spectrum in ethanol had no maxima above 210 mμ and vapour-phase chromatography showed the product to be homogeneous. The compound differed

[5] Brown and White, *J.*, 1957, 3755.
[6] Reich, Walker, and Collins, *J. Org. Chem.*, 1951, **16**, 1753.

from 1-ethylcyclohexene on the chromatogram and in its infrared spectrum. Wallach and Evans [7] record b. p. 137—138° and n_D^{20} 1·4626 for ethylidenecyclohexane.

Reduction of benzylideneacetone. Benzylideneacetone (10·0 g.) yielded, after chromatography, a colourless oil. Distillation at 189—190°/757 mm. caused some polymerisation but the bulk of the product was obtained as a colourless oil (Found: C, 90·35; H, 9·2. Calc. for $C_{10}H_{12}$: C, 90·9; H, 9·1. Calc. for $C_{10}H_{14}$: C, 89·55; H, 10·45%), $\lambda_{max.}$ (in EtOH) 250, 284, and 292·5 mμ (ε 11,750, 810, and 590). 1′,2′-Dibromobutylbenzene, obtained by saturating the product with bromine in carbon tetrachloride and crystallised several times from methanol, formed colourless needles, m. p. and mixed m. p. 71—71·5° (Found: C, 41·4; H, 4·2. Calc. for $C_{10}H_{12}Br_2$: C, 41·1; H, 4·1%).

Vapour-phase chromatography was carried out on a stationary phase of Apiezon L grease supported on fire brick (50—90 mesh) at 160°. A highly sensitive flame ionisation detector was used. With a column size of 200 × 0·45 cm., an inlet pressure 389 mm. above atmospheric, and a hydrogen flow rate of 13 ml./min. the following retention times (min.) were determined: authentic butylbenzene ($n_D^{17·5}$ 1·4906), 7·8; authentic but-1′-enylbenzene [regenerated from the dibromide; n_D^{18} 1·5424; $\lambda_{max.}$ (in EtOH) 250, 284, and 292·5 mμ (ε 18,600, 1250, and 880)], 11·0; reduction product, 7·8 and 11·0.

From the refractive index (n_D^{18} 1·5293) of the mixture after distillation, the ultraviolet spectra, and the vapour-phase chromatograms, the average value for the percentage of butenylbenzene in the mixture was 65.

We thank Professor E. R. H. Jones, F.R.S., for helpful discussion, Dr. R. W. J. Williams for a gift of ergosta-4,6,22-trien-3-one, and Mr. A. Thompson for the determination of the vapour-phase chromatograms.

Dyson Perrins Laboratory, Oxford University. [*Received, February 5th*, 1959.]

[7] Wallach and Evans, *Annalen*, 1908, **360**, 45.

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281. p-NN-*Dialkylsulphamoylbenzoic Acids.*

By A. A. Goldberg and A. H. Wragg.

p-NN-Diethylsulphamoylbenzoic acid (Long acid) and *p-NN*-di-n-propylsulphamoylbenzoic acid (Probenecid) are renal blocking agents which inhibit tubular secretion of penicillin and 4-aminosalicylic acid,[1] thereby maintaining high blood concentrations of these drugs. Recently it has been shown that Probenecid depresses the tubular reabsorption of uric acid from the glomerular filtrate and is a safe and effective uricosuric agent of value in the treatment of tophaceous gout.[2]

The methods previously described for the preparation of these substituted sulphamoylbenzoic acids, (i) oxidation of *NN*-dialkyltoluene-*p*-sulphonamides [3] and (ii) oxidation of toluene-*p*-sulphonyl chloride to *p*-carboxybenzenesulphonyl chloride and condensation of the latter with a dialkylamine,[4] are troublesome and give poor yields. The following route, however, has been found to give the compounds easily and in high yield: $Me{\cdot}C_6H_4{\cdot}SO_2{\cdot}NH_2$ (I) ⟶ $HO_2C{\cdot}C_6H_4{\cdot}SO_2{\cdot}NH_2$ (II) ⟶ $HO_2C{\cdot}C_6H_4{\cdot}SO_2{\cdot}NAlk_2$ (III). Conversion (II) ⟶ (III) is a competitive alkylation, the *N*-alkylation being irreversible and the *O*-alkylation of the carboxyl group reversible, under the strongly alkaline conditions. Much of the alkylating agent is wasted by the esterification and subsequent hydrolysis and accordingly it is necessary to add the alkyl bromide and sodium hydroxide portionwise in considerable excess with long heating periods between the additions. Jauregui [5] and Casoni [6] described the dipropyl derivatives but gave few experimental details.

[1] Boger, Martin, Gallagher, and Pitts, *J. Philadelphia Gen. Hosp.*, 1951, **1**, 51; Boger and Pitts, *Science*, 1950, **112**, 149; Israel, Mike, and Boger, *Amer. Rev. Tuberc.*, 1951, **64**, 453.
[2] Stillman, *The Practitioner*, 1957, **179**, 719.
[3] Jap. P. 3773/1952 (*Chem. Abs.*, 1954, **48**, 4002).
[4] Sato, *J. Pharm. Soc. Japan*, 1952, **72**, 74.
[5] Jauregui, Span. P. 209,068.
[6] Casoni, *Boll. Chim. farm.*, 1956, **95**, 287.

Experimental.—p-*Sulphamoylbenzoic acid.* A solution of potassium dichromate (500 g.) in sulphuric acid (400 c.c.) and water (1200 c.c.) was stirred at 100° and toluene-*p*-sulphonamide (171 g.) added portionwise during 1 hr. at such a speed that the mixture kept at 104—106°. The mixture was then stirred at a temperature just below the b. p. (108°) (to avoid foaming) for a further 40 min. and poured slowly into rapidly stirred ice and water (5 kg.). The microcrystalline precipitate of *p*-sulphamoylbenzoic acid was collected, drained, washed with cold water, and dissolved in dilute sodium hydroxide. Unchanged toluene-*p*-sulphonamide was precipitated by saturating the solution with carbon dioxide, and the product (160 g., 80%; m. p. 290—292°) recovered by acidification of the filtered solution with hydrochloric acid (Found: *M*, 202. Calc. for $C_7H_7O_4NS$: *M*, 201).

p-NN-*Diethylsulphamoylbenzoic acid.* The foregoing acid (20 g., 0·1 mol.) was dissolved in water (80 c.c.), alcohol (120 c.c.), and 10N-sodium hydroxide (20 c.c., 0·2 mol.). Ethyl bromide (20 c.c., 0·26 mol.) was added, and the solution refluxed under a 12″ condenser for 20 hr. A second addition of ethyl bromide (20 c.c.) and 10N-sodium hydroxide (20 c.c.) was made, refluxing continued for 20 hr., and then a third addition of ethyl bromide (20 c.c.) and 10N-sodium hydroxide (20 c.c.) made and refluxing continued for a further 20 hr. Solvent (30 c.c.) was distilled off and the residual liquor, containing much ethyl *p*-diethylsulphamoylbenzoate, was diluted with alcohol (30 c.c.) and 10N-sodium hydroxide (52 c.c.) and refluxed for 2 hr. Solvent (120 c.c.) was distilled off at reduced pressure, the residue diluted with water (500 c.c.), and the solution filtered and extracted with benzene (100 c.c.) to remove any unhydrolysed ester. The aqueous solution was filtered (charcoal) and the product (19·8 g.; m. p. 186—190°) precipitated with 5N-hydrochloric acid. Recrystallisation from 50% alcohol (170 c.c.) gave *p*-diethylsulphamoylbenzoic acid (18·0 g., 70%) as stout needles, m. p. 194° (Found: *M*, 256; N, 5·7; S, 12·6%. Calc. for $C_{11}H_{15}O_4NS$: *M*, 257; N, 5·5; S, 12·5%).

p-NN-*Di-n-propylsulphamoylbenzoic acid.* A solution of *p*-sulphamoylbenzoic acid (20·1 g.) in water (120 c.c.), alcohol (180 c.c.) and 10N-sodium hydroxide (18 c.c., 0·18 mol.) was refluxed with n-propyl bromide (25·2 c.c., 0·27 mol.) for 24 hr. Four further additions of propyl bromide (25 c.c.) and 10N-sodium hydroxide (18 c.c.) were made, the solution being refluxed for 24 hr. after each addition. 10N-Sodium hydroxide (80 c.c.) was added, and the solution refluxed for 4 hr. to hydrolyse the ester and then evaporated to near dryness at reduced pressure. Boiling water (500 c.c.) was added, the solution filtered (charcoal), and the *p*-dipropylsulphamoylbenzoic acid (26·6 g.; m. p. 190—192°) precipitated by strongly acidifying the solution with hydrochloric acid. The crude dried acid was extracted for 40 hr. in a Soxhlet apparatus with benzene in order to dissolve out dialkylated acid from the insoluble unalkylated and monoalkylated acids. The benzene extract was made up to 700 c.c. with benzene in order to obtain a clear solution at the b. p. and then cooled. The precipitate, on crystallisation from benzene-alcohol, gave dipropylsulphamoylbenzoic acid (20·8 g., 73%) as needles, m. p. 196—198° (Found: *M*, 286. Calc. for $C_{13}H_{19}O_4NS$: *M*, 285).

p-NN-Di-n-butylsulphamoylbenzoic acid was obtained in like manner in 70% yield as needles, m. p. 162° (Found: *M*, 314. Calc. for $C_{15}H_{23}O_4NS$: *M*, 313).

ANGLO ESTATE, SHEPTON MALLET, SOMERSET. [*Received, September 2nd,* 1959.]

282. *Synthesis of Fulvene by Thiele's Method.*

By H. J. F. ANGUS and D. BRYCE-SMITH.

THIEC and WIEMANN [1] recently reported the isolation and some properties of fulvene prepared by Thiele's general method, *viz.*, the base-catalysed condensation of cyclopentadiene and aldehydes or ketones.[2] In repeated experiments we have been unable to duplicate this preparation, the yields of fulvene being consistently less than 0·2%. Detailed instructions kindly provided by Professor Wiemann proved no better in our hands. Variations in reaction temperature, base, and medium have been examined. In

[1] Thiec and Wiemann, *Bull. Soc. chim. France*, 1956, 177.
[2] Thiele, *Ber.*, 1900, **33**, 672; Thiele and Balhorn, *Annalen*, 1906, **348**, 1.

forty-six experiments, the highest yield was 0·5%, although in only one case (pyridine being used as base) was the yield zero. Our most favourable conditions are described below. We are unable to explain the repeated failure of Thiec and Wiemann's method, but feel that unrevealed factors are in operation. In view of the theoretical importance of their results, some duplication might be desirable.

Experimental.—Cyclopentadiene (10 g.), formaldehyde (12 ml.; 40% aqueous), and anhydrous potassium carbonate (4 g.) were added in that order to ethanol (200 ml., 99%) at room temperature (20—25°), and the mixture was stirred under nitrogen for 18 hr. Water (200 ml.) was added, and the mixture brought to pH 6 with 2% aqueous acetic acid. The product was extracted by cyclohexane (3 × 100 ml.). The combined extracts were washed twice with water (2 × 100 ml.) and dried ($MgSO_4$). Pentane (40 ml.) was added and the mixture was fractionally distilled, the forerun, b. p. <80°, being rejected. Yellow material (286 ml.), b. p. 80—81°, was obtained. The optical density at 360 mμ was 0·565, indicating the presence of 0·06 g. of fulvene.[1]

We thank Esso Research Ltd. for a maintenance grant (to H. J. F. A.).

THE UNIVERSITY, READING. [*Received, September 28th*, 1959.]

283. *The Wax of the Silk-worm Ecdysis Skin. Part I. The Fatty Acids.*

By EL S. AMIN.

COCOONS of the silk-worm moth include the last larval ecdysis skin. The author collected 300 g. of that skin, extracted it with chloroform, and got a green wax. This was chromatographed over aluminium oxide, and the lowest main band was saponified. The resultant acids were nearly neutralised and warmed with 4-*p*-nitrophenylazophenacyl bromide [1] in ethanol. Chromatography, partition chromatography, and crystallisation gave sharp-melting tetradecanoic, hexadecanoic, and octadecanoic esters The author used cellulose columns in preference of kieselguhr in chromatography, since they are relatively simple to prepare, give reproducible results, and are eluted faster. Ester mixtures were also eparated on filter paper [4,5] (see Table).

Experimental.—Evaporations were under reduced pressure at 50°. M. p.s were taken on the Kofler microscope stage. Chromatography was on columns of cellulose powder treated with dichlorodimethylsilane,[2] also on Whatman No. 1 filter paper treated with dichlorodimethylsilane [3] by the descending method at room temperature. Nonane–dimethylformamide–nitromethane (15 : 3 : 1 v/v) was used as eluant for untreated cellulose, dimethylformamide–water–nonane (9 : 1 : 3 v/v) and 80% acetone–nonane (5 : 1 v/v) for treated cellulose.

Almost complete separation was achieved of octadecanoic–hexadecanoic, hexadecanoic–tetradecanoic, tetradecanoic–dodecanoic, and dodecanoic–decanoic esters. The average relative R values are tabulated.

4-*p*-Nitrophenylazophenacyl Ester	Dimethylformamide–water–nonane (9 : 1 : 3 v/v) $R_{octanoic}$	Nonane–dimethylformamide–nitromethane (15 : 3 : 1 v/v) $R_{octadecanoic}$	80% Acetone–nonane (5 : 1 v/v) $R_{dodecanoic}$
Octanoic	1·0		
Decanoic	0·92	0·31	
Dodecanoic	0·76	0·48	1·0
Tetradecanoic	0·65	0·54	0·5
Hexadecanoic	0·48	0·7	0·3
Octadecanoic	0·32	1·0	0·2

[1] Amin and Hecker, *Chem. Ber.*, 1956, **89**, 1496.
[2] Amin, *J.*, 1957, 3764.
[3] Theodore, Kritchevsky, and Tiselius, *Science*, 1951, **114**, 299.
[4] Neher and Wettstein, *Helv. Chim. Acta*, 1952, **35**, 276.
[5] Consden, Gordon, and Martin, *Biochem. J.*, 1944, **38**, 224.

Extraction of ecdysis skin. Larval skin (300 g.) was dried for 16 hr. at 60—70°/10—15 mm. and minced in chloroform (31°) for 24 hr. The crude extract was decanted from the sludge, washed with 20% sulphuric acid, filtered, washed successively with water, sodium carbonate solution, and water, dried (Na_2SO_4), and evaporated to dryness (yield 4·8 g., 1·6%).

Chromatography of the wax. The deep green extract was dissolved in benzene (30 ml.) and filtered through activated alumina. Nine bands were produced. The lowest band (orange) was eluted with benzene, giving a pale orange substance (4·0 g.), and four recrystallisations from ethanol gave colourless crystals (3·4 g., 1·1% of the larval skin), m. p. 54—58°.

Saponification and isolation of the fatty acids.[6] The wax (3·4 g.) and benzene (50 ml.) at 40° were added with stirring to 5% ethanolic potassium hydroxide (170 ml.) and the mixture was stirred at 40° for 24 hr. Then 96% ethanol (150 ml.) containing calcium chloride (17·5 g.) was added and the whole was boiled for a further 2 hr. The mixture was then filtered hot. The residual calcium soaps were extracted with boiling alcohol (3 × 500 ml.) and then with 500 ml. of boiling acetone, and then dried (yield 2·5 g.).

The filtrates were evaporated to dryness, and the residue was boiled in benzene (60 ml.) for 3·5 hr. with sodium ethoxide (1 g. in 24 ml. of ethanol). Calcium chloride (1·5 g. in 12 ml. of ethanol, was added and boiling continued for 2 hr. The mixture was filtered hot and the residue washed with boiling alcohol (50 ml.) and boiling acetone (50 ml.) and then dried (yield 0·3 g.).

The combined calcium salts (2·8 g.) were refluxed with glacial acetic acid (100 ml.) until the solution was clear and the mixture was poured into cold water and extracted with ether. After drying (Na_2SO_4), the ethereal extracts were evaporated (yield 300 mg.; m. p. 66—70°).

Separation of the fatty acids. The material (300 mg.) was nearly neutralised with ethanolic sodium hydroxide and mixed with 4-*p*-nitrophenylazophenacyl bromide (0·5 g.) and refluxed for 2 hr. at 50°. The mixture was then diluted with water (100 ml.) and extracted with benzene (100 ml.). The benzene extract was washed with 20% sulphuric acid, filtered, washed in turn with water, sodium carbonate, and water, and filtered through an alumina column (yield 0·5 g.). Examination on the paper chromatogram indicated tetradecanoic (R_O 0·65), hexadecanoic (R_O 0·48) and octadecanoic esters (R_O 0·32).

Quantitative determination gave 10%, 30%, and 45% of the above mentioned esters respectively.

Another portion (0·2 g.) was chromatographed and the substances of the bands were eluted and recrystallised giving the tetradecanoic ester (15 mg.), m. p. and mixed m. p. 132° (Found: N, 8·6. Calc. for $C_{28}H_{37}O_5N_3$: N, 8·52), the hexadecanoic ester (36 mg.), m. p. and mixed m. p. 132° (Found: N, 8·1. Calc. for $C_{30}H_{41}O_5N_3$: N, 8·0%), and the octadecanoic ester (40 mg.), m. p. and mixed m. p. 132° (Found: N, 7·5. Calc. for $C_{32}H_{45}O_5N_3$: N, 7·6%).

ALEXANDRIA UNIVERSITY, EGYPT. [*Received, October 2nd,* 1959.]

[6] Chibnall, Latner, Williams, and Ayre, *Biochem. J.*, 1934, **28**, 313.

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284. *Incorporation of Tritium into* trans-*Stilbene by the Gas Exposure Method.*

By G. G. CAMERON, N. GRASSIE, and S. J. THOMSON.

IN preparing an organic compound labelled with tritium, three possible methods are available: (*a*) chemical synthesis, *e.g.*, hydrogenation, (*b*) recoil labelling[1] by tritons produced from neutron irradiation of a compound in admixture with a lithium salt, and (*c*) the gas exposure method, in which the compound is simply sealed into a vessel with tritium gas.[2,3] In some cases method (*a*) is not possible, or is laborious. The appeal of the last two lies in their apparent simplicity; this is especially true of method (*c*), which requires simple apparatus. Methods (*b*) and (*c*), however, have a serious limitation, *viz.*, that considerable radiation damage can result, so that the product is not radiochemically

[1] Rowland and Wolfgang, *Nucleonics*, 1956, **14**, No. 8, 58.
[2] Wilzbach, *J. Amer. Chem. Soc.*, 1957, **79**, 1013.
[3] *Nucleonics*, 1958, **16**, No. 3, 62.

pure. This is well illustrated in our preparation of tritiated stilbene by the third method, for use as a tracer in copolymerisation experiments.

The procedure was similar to that previously described,[2,3] although a lower gas pressure was used. Pure, finely-powdered *trans*-stilbene (2 g.) was exposed in a tube (the whole being thoroughly degassed) for 16 days to 2 curies of tritium. The product was recrystallised from ethanol and had m. p. 125° (cf. stilbene 124°).

The tritium content was estimated as follows.[4] The sample was diluted with inactive stilbene and burned to carbon dioxide and water, and the water passed over magnesium at 500° to produce hydrogen. Tritium in this was counted in a Geiger–Muller tube. The specific activity found for the original product was $5{\cdot}16 \times 10^6$ μc/mole.

The radiochemical purity of the product, diluted 10^6 times with inactive *trans*-stilbene, was investigated by means of chromatography on Brockmann Grade III alumina, benzene–light petroleum (b. p. 40—60°) (4 : 1, v/v) being used as solvent. The results, expressed as μc/mole, were: (*a*) tritiated product 5·13; (*b*) first fraction from chromatography 30·20; (*c*) second fraction, 1·07; (*d*) third fraction, 0·43. The second fraction was further divided into two fractions with activities of 0·26 and 0·11.

The product was thus far from being radiochemically pure; and column chromatography was clearly not suitable for achieving purity.

Zone melting was then investigated but this also gave fractions which were not uniform in radioactivity.

A radiochemically-pure product was finally produced by passing the undiluted product through a sequence of chemical reactions likely to isolate only *trans*-stilbene. This was done by preparing the dibromide, which was recrystallised from hot xylene, and debrominating the product with zinc in boiling ethanol. The resulting *trans*-stilbene was recrystallised from ethanol and its radiochemical purity checked as before by chromatography. The first fraction had an activity of $3{\cdot}85 \times 10^4$ μc/mole, the second of $3{\cdot}94 \times 10^4$. The second fraction was divided by chromatography into two fractions both having an activity of $3{\cdot}94 \times 10^4$ μc/mole. Thus it seemed that a radiochemically-pure specimen had been produced. Such a multi-stage confirmatory test is generally considered to be adequate proof of radiochemical purity.[5]

The activity of the purified stilbene ($3{\cdot}94 \times 10^4$ μc/mole), compared with the original ($5{\cdot}16 \times 10^6$), showed that considerable active impurity was present in the latter. The mass yield of pure, active, *trans*-stilbene was about 45%, although this could be increased. The very great difference in activity between the crude and purified *trans*-stilbene could be due to the addition of tritium at the ethylenic double bond. This has been shown to take place in a number of unsaturated compounds.[6] It may also account for the low final activity, since toluene and benzoic acid under similar treatment gave activities between 10 and 25 mc/g.[3]

For compounds such as stilbene, the chemical preparation of labelled material is therefore probably more satisfactory than exposure labelling. This has been done by Bernstein *et al.*,[7] who obtained pure, tritiated *trans*-stilbene, of activity $6{\cdot}48 \times 10^7$ μc/mole, with a mass yield of 67%.

We conclude that the ease of labelling by exposure is deceptive, and that, if the method is used, strict precautions must be taken in order to isolate radiochemically-pure products.

G. G. C. thanks the Salters' Company for a Fellowship.

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[4] Walton and Thomson, *Trans. Faraday Soc.*, 1957, **53**, 821.
[5] Rosenblum and Meriwether, "Proceedings of the Symposium on Advances in Tracer Applications of Tritium," New England Nuclear Corp., 575 Albany Street, Boston 18, Mass., Oct. 31st, 1958, p. 3.
[6] Dutton and Nystrom, *ibid.*, p. 8.
[7] Bernstein, Bennett, Fields, and Farmer, *Nucleonics*, 1953, **11**, No. 2, 64.