NOTES.

Observations on the Synthesis of [8-^{13}C]*Adenine.*

By MAXWELL GORDON.

[Reprint Order No. 4657.]

IN the synthesis of [8-^{14}C]adenine by cyclization of 4 : 6-diamino-5-formamidopyrimidine in formamide, Cavalieri and Brown (*J. Amer. Chem. Soc.*, 1949, **71**, 2246) originally noted a 75% exchange of the labelled formyl group with the unlabelled formic acid from the formamide. Clark and Kalckar (*J.*, 1950, 1029) reported that this exchange could be reduced to about 20% by the use of 4-formylmorpholine as the cyclizing solvent. Abrams and Clark (*J. Amer. Chem. Soc.*, 1951, **73**, 4609) were unable to repeat Clark and Kalckar's work, and postulated a diformamidopyrimidine which could cyclize in either direction to give a theoretical maximum of 50% isotope incorporation.

We have made labelled adenine by Clark and Kalckar's procedure using ^{13}C, thus avoiding any counting error such as that postulated by Abrams and Clark. We have

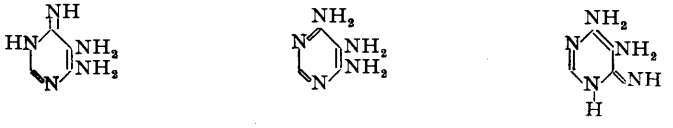
Expt. no.	$H{\cdot}CO_2H$ (atom % excess of ^{13}C)	Adenine (atom % excess of ^{13}C)	Wt. yield * (%, based on formate)	Isotope yield (%)
1	42·5	6·67	36·1	78·5
2	42·5	6·45	28·4	76
3	42·5	6·79	22·0	80

* Syntheses on a 1 mmole scale.

succeeded in carrying out repeated syntheses in which the incorporation of isotope into the adenine was in excess of 75% (cf. Table). Thus our results support those of Clark and Kalckar. Data from chemical reactivity and spectra show that the 4- and the 6-amino-group have some imine character and are less reactive than the 5-amino-group. Hence all these formamido-groups would not be expected to be equally reactive, as would

be required by Abrams and Clark's postulate. Indeed, to our knowledge, no diformyl-4 : 5 : 6-triaminopyrimidine has been isolated.

Some of the potential tautomeric forms of 4 : 5 : 6-triaminopyrimidine are shown below to illustrate the partial imine character of the 4- and the 6-amino-group :



Abrams and Clark have speculated that the discrepancies between their own results and those of Clark and Kalckar were due to (1) the use of the formamido-compound as the sulphate rather than as the hydrochloride, and (2) the fact that direct plating rather than combustion of ^{14}C-samples to barium carbonate might have been used to prepare ^{14}C-compounds for counting. Regarding the first possibility we have no information. The second speculation is improbable for two reasons. First, our work was done with ^{13}C with which this counting problem does not arise, and, secondly, results on determination of radioactivity can be obtained by direct plating of soluble samples which are as good as those obtained by combustion before counting—with a considerable saving of time. One method of obtaining reproducible direct glass plates involves wiping the plates with a trace of detergent before preparation of the sample (Gordon, Virgona, and Numerof, *Analyt. Chem.*, in the press).

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ORGANIC CHEMISTRY DEPARTMENT, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, S. KENSINGTON, LONDON, S.W.7.
[Present address : SQUIBB INSTITUTE FOR MEDICAL RESEARCH, NEW BRUNSWICK, NEW JERSEY, U.S.A.] [*Received, September 15th*, 1953.]

2-Acetamido-7-methoxyfluorene.

By JOHN H. WEISBURGER and ELIZABETH K. WEISBURGER.

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2-ACETAMIDO-7-HYDROXYFLUORENE, although a metabolite of the carcinogen 2-acetamidofluorene (Bielschowsky, *Biochem. J.*, 1945, **39**, 287 ; Weisburger *et al.*, *Fed. Proc.*, 1953, **12**, 287), is itself not carcinogenic (Bielschowsky, *Brit. Med. Bull.*, 1947, **4**, 382 ; Hoch-Ligeti, *Brit. J. Cancer*, 1947, **1**, 391). A similar situation occurs with 8-hydroxy-3 : 4-benzopyrene (Cook and Schoental, *ibid.*, 1952, **6**, 400), but 8-methoxy-3 : 4-benzopyrene is as active as 3 : 4-benzopyrene. We have therefore prepared 2-acetamido-7-methoxyfluorene for biological test ; the procedures for preparation of the intermediates are improvements on those previously recorded (Ruiz, *Anal. Asoc. Quím. Argentina*, 1928, **16**, 170 ; Bielschowsky, *loc. cit.*, 1945 ; Goulden and Kon, *J.*, 1945, 930 ; Gutmann, *J. Amer. Chem. Soc.*, 1952, **74**, 836).

Experimental.—2 : 7-*Dinitrofluorene.* Fluorene was dinitrated as described by Courtot and Moreaux (*Compt. rend.*, 1943, **217**, 453) with this modification : The insoluble 2 : 7-dinitro-isomer was filtered off on a sintered-glass funnel from the warm reaction mixture, boiled in glacial acetic acid (2 l./100 g.) for 1 hr., and filtered off hot. This process was repeated twice to leach out any 2 : 5-isomer. The resulting material melted at 340° (block).

2-*Amino*-7-*nitrofluorene.* This compound was prepared by Cislak and Hamilton's method (*J. Amer. Chem. Soc.*, 1931, **53**, 746). Best yields (80—90%) resulted when the hydrogen sulphide was bubbled into the mixture at a fast rate and the reaction time limited to 2 hr. The toluene-*p*-sulphonamide sintered at 202° but melted at 215—215·5° after chromatography on alumina and recrystallisations from benzene and acetic acid (Found : C, 62·9 ; H, 4·35 ; N, 7·5. Calc. for $C_{20}H_{16}O_4N_2S$: C, 63·1 ; H, 4·2 ; N, 7·4%). Bell and Mulholland (*J.*, 1949, 2020) reported m. p. 202°.

2-*Toluene*-p-*sulphonamido*-7-*aminofluorene*. Reduction of the nitro-compound (2 g.) by zinc dust (4 g.) and calcium chloride (1 g.) in refluxing 80% ethanol (120 ml.) for 3 hr. gave a *product* (1·3 g.), m. p. 160—165°. Two recrystallisations from 50% ethanol raised the m. p. to 170—171° (Found N, 8·1. $C_{21}H_{18}O_2N_2S$ requires N, 8·0%).

2-*Acetamido*-7-*hydroxyfluorene*. 2-Amino-7-nitrofluorene (59 g.), dissolved in acetic acid (660 ml.) and 10N-sulphuric acid (155 ml.) and cooled, was diazotised with sodium nitrite (22 g.) in water (33 ml.). Urea (5·5 g.) in water (22 ml.) and cold 2·5N-sulphuric acid (650 ml.) was added after 1 hr. The suspension was added in portions to a boiling, mechanically stirred mixture of xylene (440 ml.) and 2·1N-sulphuric acid (1700 ml.) (the xylene prevented separation of tars), and the mixture boiled for 30 min. longer and then cooled. Yellow granules of 2-*hydroxy*-7-*nitrofluorene* (59 g.), m. p. 239—246°, were obtained. Samples crystallised from xylene, acetic acid, ethanol, or dioxan melted at 248—250° (cf. Bielschowsky, *loc. cit.*, 1945).

Reduction to the amine and acetylation, essentially by Bielschowsky's method, gave 2-*acetamido*-7-*hydroxyfluorene* (48 g.), m. p. 230—232° (transition pt. 216°) (Found: C, 75·4; H, 5·4; N, 5·8. Calc. for $C_{15}H_{13}O_2N$: C, 75·3; H, 5·5; N, 5·9%). The ultra-violet absorption, determined in ethanol (5 mg./l.) on a Cary automatic recording instrument, showed: min., 246 mμ (ε 1100); max. 293 mμ (ε 29,800); infl. 326 mμ (ε 11,500). The *acetoxy*-derivative, obtained by reaction with acetic anhydride (8 ml./1 g.) and fused sodium acetate (0·3 g./g.), melted at 137° after crystallisation from benzene and benzene–light petroleum (Found: C, 70·9; H, 5·7; N, 4·4; Ac, 30·8. $C_{17}H_{15}O_3N$ requires C, 70·9; H, 5·4; N, 5·0; Ac, 30·5%).

2-*Acetamido*-7-*methoxyfluorene*. 2-Acetamido-7-hydroxyfluorene (35·2 g.) in 1·1N-potassium hydroxide (530 ml.) was methylated at 10° with methyl sulphate (40 ml.), then stirred while being allowed to warm to 30°. After 2 hr. methyl sulphate (8 ml.) and solid potassium hydroxide (10 g.) were added and stirring continued for another hour. The precipitated material (32·5 g.), m. p. 199—201°, was filtered off and washed with water until the washings were neutral. A small amount (0·9 g.) of impure starting material was recovered from the filtrate by acidification.

The product was dissolved in hot ethanol (1250 ml.) (3 g. of Norit); concentration of the filtrate to about 700 ml. afforded the *ether* as shiny leaflets (24 g.), m. p. 204—205°. A sample (1·43 g.), recrystallised from 65% ethanol (75 ml.), had m. p. 204—206° (1·25 g.) Found: C, 75·5; H, 6·2. $C_{16}H_{15}O_2N$ requires C, 75·9; H. 6·0%). The ultra-violet absorption (8 mg./l.; EtOH) showed: min. 244 mμ (ε 1580), max. 293 mμ (ε 29,500); infl. 280 (ε 25,300) and 324 mμ (ε 11,500). Hydrolysis with 1 : 1 ethanol–concentrated hydrochloric acid gave 2-amino-7-methoxyfluorene, identical with that obtained by reduction of the nitro-compound (see below).

2-*Methoxy*-7-*nitrofluorene*. To a refluxing solution of 2-hydroxy-7-nitrofluorene (1·16 g.) in 0·4N-potassium hydroxide (35 ml.) and methanol (10 ml.) methyl sulphate (1 ml.) in methanol (5 ml.) was added with stirring. After 15 min. 2·3N-potassium hydroxide (6 ml.), methanol (10 ml.), and methyl sulphate (1·5 ml.) were added and the mixture was refluxed for 2·5 hr. The volume was reduced by approx. one-half and the solution was diluted with water to 100 ml. and cooled. The resulting precipitate was filtered off and washed until neutral, yielding a yellow powder (1·04 g.), m. p. 204—209°. From the acidified filtrate starting material (151 mg.), m. p. 244—248°, was recovered. The *product*, purified by passage in benzene through alumina, (yield, 610 mg.; m. p. 214—218°), crystallised from acetic acid as lemon-yellow needles (515 mg.), m. p. 216—219° (Found: C, 69·9; H, 4·8. $C_{14}H_{11}O_3N$ requires C, 69·7; H, 4·6%).

2-*Amino*-7-*methoxyfluorene*. A mixture of the nitro-derivative (502 mg.), zinc dust (1·7 g.), calcium chloride (140 mg.), and 80% ethanol (24 ml.) was refluxed with stirring for 2 hr., then filtered with suction into concentrated hydrochloric acid (1 ml.). This solution was boiled down to a small volume and cooled; the crystalline precipitate was filtered off and washed with 3N-hydrochloric acid. The yellowish solid was refluxed briefly in 0·06N-hydrochloric acid (50 ml.), and the solution was filtered hot. The colourless filtrate was made alkaline with ammonia, giving a white precipitate of the *amine* (287 mg.), m. p. 189—190° (Found: C, 79·5; H, 6·2. $C_{14}H_{13}ON$ requires C, 79·6; H, 6·2%). The acetyl derivative (prepared by acetic anhydride in benzene) had m. p. 205—206° unchanged by crystallisation.

The authors are indebted to Mr. James Williams and Miss Rita McCallum for the microanalyses.

NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE,
U.S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE,
BETHESDA 14, MARYLAND, U.S.A. [*Received, October 3rd*, 1953.]

2 : 3-*Dimethyl*-1 : 4-*naphthaquinol Bis*(*dihydrogen Phosphate*).

By D. H. Marrian.

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Since the compound named in the title appeared to possess interesting biological properties (cf., *e.g.*, Mitchell and Simon-Reuss, *Brit. J. Cancer*, 1952, **6**, 317), its preparation and characterisation are described. All evaporations took place *in vacuo* in nitrogen.

Experimental—2 : 3-Dimethyl-1 : 4-napthaquinone (Kruber, *Ber.*, 1929, **62**, 3046) was shaken in methyl alcohol with hydrogen in the presence of reduced platinum oxide. The quinol obtained on evaporation was phosphorylated by the procedure described by Roche Products Ltd. (B.P. 593,480; see also Friedmann, Marrian, and Simon-Reuss, *Brit. J. Pharmacol.*, 1948, **3**, 263). 2 : 3-*Dimethyl*-1 : 4-*naphthaquinol di*(*barium phosphate*) *pentahydrate* crystallised in colourless prisms when its aqueous solution was heated to 90° in the water-bath with stirring. The salt was filtered off hot, washed with a little hot water, alcohol, and ether, and dried *in vacuo* (yield 77%) (Found, in material dried at room temperature: C, 20·0; H, 2·7; P, 8·55; loss at 120° *in vacuo*, 11·6. $C_{12}H_{10}O_8P_2Ba_2,5H_2O$ requires C, 20·4; H, 2·8; P, 8·75; $5H_2O$, 12·7%).

The barium salt (17·2 g.) was added to a solution of sodium sulphate (15·6 g. of decahydrate) in water (80 ml.) and shaken in the cold room overnight. The solution was filtered with the aid of "Hyflo" and gradually treated with 2 volumes of alcohol. The crystalline precipitate was filtered off, and washed once with aqueous alcohol (33% of EtOH), once with alcohol, and once with ether. 2 : 3-*Dimethyl*-1 : 4-*naphthaquinol bis*(*disodium phosphate*) formed colourless hydrated needles after drying off *in vacuo*. (13·4 g., 69%) (Found, in material dried in *vacuo* C, 18·2; H, 6·2; P, 7·15; loss at 60° *in vacuo*, 46·7. $C_{12}H_{10}O_8P_2Na_4,20H_2O$ requires C, 18·1; H, 6·3; P, 7·8; $20H_2O$, 45·2%).

2 : 3-*Dimethyl*-1 : 4-*naphthaquinol bis*(*dihydrogen phosphate*) was obtained by passing a solution of the dibarium salt through a column of Zeo-Karb 215 (H form). The colourless solution was evaporated, leaving a colourless solid which recrystallised from a little hot water as prisms, m. p. 260° (decomp.) (Found, in material dried at room temperature: C, 39·5; H, 4·1; P, 17·3%; equiv., 83·5. $C_{12}H_{14}O_8P_2,H_2O$ requires C, 39·4; H, 4·4; P, 16·9%; equiv., 91·5).

In 0·01N-hydrochloric acid the above compounds show maxima at 230—231 (ε 64,200) and 290—291 mμ (ε 5450) (measured on a Unicam S.P. 500 spectrophotometer). They may be quickly characterised by the following ratios of their absorptions at the wave-lengths (mμ) stated (pH2): 250/260 = 0·61; 280/260 = 2·50; 290/260 = 2·68; 310/260 = 1·13.

The author is grateful to Roche Products, Welwyn Garden City, Herts, and especially to Dr. F. Bergel and Dr. A. L. Morrison for their interest.

Department of Radiotherapeutics,
The University, Cambridge.

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Tetramethylammonium Amalgam.

By G. B. Porter.

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Tetramethylammonium amalgam was first isolated by McCoy and Moore (*J. Amer. Chem. Soc.*, 1911, **33**, 273), who reported that the organic ion NMe_4^+ displaces from aqueous solution Cu^{2+}, NH_4^+, Na^+ and K^+, and to a slight extent Rb^+ and Cs^+, that the amalgam decomposes rapidly (with inflation) above 0°, one of the products being trimethylamine, and that it reacts violently with water, forming hydrogen, black colloidal mercury, and tetramethylammonium hydroxide. By filtering off and weighing the colloidal mercury and titrating the filtrate with standard hydrochloric acid, they estimated the ratio Hg : NMe_4 for different samples. This ratio was not constant and gave no definite indication of compound formation.

The amalgam has now been prepared by a similar method and the above observations have been confirmed. It was thought that if free methyl radicals are liberated during thermal decomposition, these should be detectable under suitable conditions: $\cdot NMe_4 \longrightarrow NMe_3 + Me\cdot$. By using Paneth's "rapid flow" technique (*Ber.*, 1929, **62**, 1335) with tellurium mirrors (Rice and Glasebrook, *J. Amer. Chem. Soc.*, 1934, **56**, 2472), minute

concentrations of methyl radical were detected in the products of dissociation under reduced pressure.

Pure dry nitrogen was passed at 2 mm. pressure over the frozen amalgam (containing traces of the alcohol used in preparation) and through a long quartz tube (50 × 0·5 cm.), in which a tellurium mirror was condensed at *A* (the mouth of the tube). The amalgam was allowed to melt and decompose, and the tube was heated with a micro-burner (non-luminous flame) at *C* (about 40 cm. from *A*), where a faint, translucent white deposit was formed. When the tube was heated at *B* (about 30 cm. from *A*), another faint deposit appeared there, and the methyl produced at *B* began to remove the mirror at *C*. The mirrors at *B* and *C* were tested for tellurium and mercury; only the former was found. The only product found in the cold traps after each experiment was an alcoholic solution of trimethylamine.

Experimental.—The amalgam was prepared, in an apparatus similar to that described by McCoy and Moore (*loc. cit.*), by electrolysis of a solution of 5 g. of recrystallised tetramethylammonium chloride in 100 ml. of absolute alcohol at −15° (CO_2–acetone) with a current of 0·4 amp. for 15 min., between a silver anode and a redistilled mercury cathode. The product was removed through a wide-bore tap beneath the electrolytic cell, after excess of mercury had been run off, and stored at −78°.

The amalgam softens at −28° to a semi-solid paste, and is fairly stable at 0° when freshly prepared, but after it has been kept for a few days it begins to decompose at the m. p. and the decomposition is usually complete at 0°. It is difficult to remove the last traces of alcohol, with which it slowly reacts.

It was not possible to determine the properties of the amalgam in more detail (*e.g.*, electrode potential), because of its instability and variable composition.

The author thanks Professor E. C. Baughan for advice and help.

ROYAL MILITARY COLLEGE OF SCIENCE,
SHRIVENHAM, WILTS.

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The Addition of Some Acyl Hypobromites to Allyl Bromide.

By W. G. H. EDWARDS and R. HODGES.

[Reprint Order No. 4713.]

ISRAEL, TUCK, and SOPER (*J.*, 1945, 548) postulated the formation of acetyl hypobromite in the bromination of anisole by *N* : 2 : 4 : 6-tetrabromoacetanilide in acetic acid solution. Bromination of allyl bromide by the same reagents gave rise to an acetate $C_5H_8O_2Br_2$ (Mitchell and Soper, unpublished), and when trichloroacetic acid was replaced by acetic acid in this reaction, which was catalysed by pyridine, we obtained a mixture of a dibromopropyl trichloroacetate and a dibromopropanol.

When pre-formed acetyl, butyryl, and benzoyl hypobromites were added to allyl bromide, 2 : 3-dibromopropyl esters were isolated in yields of 60—85%. The products were identified by comparison of their infra-red spectra with those of authentic specimens, and, after catalytic debromination with hydrogen and palladised strontium carbonate, with the spectra of propyl esters previously reported (Edwards and Hodges, *J.*, 1953, 3427). The nature of the products was the same in the presence or absence of peroxides, and whether carbon tetrachloride or nitrobenzene was used as solvent.

Abbott and Arcus (*J.*, 1952, 1516) showed that 2-bromo-1-phenylethyl acetate (I) was produced by addition of acetyl hypobromite to styrene. We extended this reaction to *p*-methylstyrene and benzoyl hypobromite, but were able to isolate in a pure state only one product, which appeared to be a benzoate, $C_{16}H_{14}O_2$. This probably has the structure (II), being formed from 2-bromo-1-*p*-tolylethyl benzoate by loss of hydrogen bromide.

(I) $CH_2Br{\cdot}CHPh{\cdot}OAc$ $C_6H_4Me{\cdot}C(OBz){:}CH_2$ (II)

Experimental.—Bromination of allyl bromide with N : 2 : 4 : 6-*tetrabromoacetanilide.* To a solution of *N* : 2 : 4 : 6-tetrabromoacetanilide (22·6 g.) in chloroform (100 c.c.) were added trichloroacetic acid (24·0 c.c. of a 2·08M-solution in chloroform), pyridine (4·05 c.c.), and allyl bromide (4·3 c.c.). After 1½ hr., the precipitated tribromoacetanilide was filtered off, the chloroform removed by distillation, and the residue fractionally distilled under reduced pressure.

The fraction of b. p. 100°/1 mm. (1·5 g.) was redistilled for determination of its infra-red spectrum (chief maxima at 7·5, 8·7, 9·0, 9·8, 11·8, and 13·3 μ, with a band at 2·8 μ characteristic of the OH group of 2 : 3-dibromopropanol).

Reaction of acyl hypobromites with allyl bromide. (1) 2 : 3-Dibromopropyl butyrate. Silver butyrate (19·5 g., 0·12 mole) was suspended in dry carbon tetrachloride (250 c.c.), and about 50 c.c. of the solvent were evaporated to remove water. The cooled suspension was treated gradually with shaking at −10° with bromine (5·12 c.c., 0·1 mole), and shaking was continued until the colour of the bromine had faded to a pale yellow (3 min.). To the resulting solution of butyryl hypobromite was added at once pure allyl bromide (8·43 c.c., 0·1 mole), the mixture was shaken for 10 min., and the silver salts removed by filtration. The filtrate was shaken with saturated sodium hydrogen carbonate solution, washed with water, dried (Na_2SO_4), and evaporated. The residue was distilled under reduced pressure, and afforded 2 : 3-dibromopropyl butyrate, b. p. 118°/6·5 mm. (24·6 g., 85%).

(2) 2 : 3-Dibromopropyl benzoate and acetate. These were similarly prepared from silver benzoate and acetate respectively. 2 : 3-Dibromopropyl benzoate had b. p. 165°/3 mm., and the acetate, b. p. 68°/3 mm.

Addition of benzoyl hypobromite to p-*methylstyrene.* In the above method, silver benzoate (0·12 mole) and *p*-methylstyrene (0·1 mole) were used in place of silver butyrate and allyl bromide. Distillation of the addition product under reduced pressure gave a yellow oil, b. p. 150—170°/0·2 mm., which deposited crystals. From ethanol these formed colourless needles, m. p. 96° (Found : C, 80·5; H, 6·0. Calc. for $C_{16}H_{14}O_2$: C, 80·65; H, 5·9%). The infra-red absorption spectrum showed strong bands at 5·8, 7·8, and 9·0 μ, indicating the presence of a benzoate group.

The authors express their appreciation of the interest and advice of Professor F. G. Soper during the investigation, and thank Dr. A. D. Campbell for microanalyses. The assistance of the Mellor Fund is gratefully acknowledged.

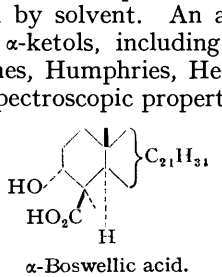
OTAGO UNIVERSITY, DUNEDIN, NEW ZEALAND. [*Received, October 10th,* 1953.]

Effect of Solvent on the Optical Rotation of Some Steroid α-Ketols.

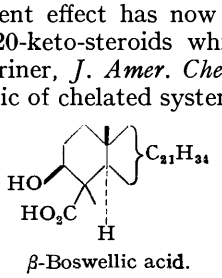
By J. K. NORYMBERSKI.

[Reprint Order No. 4768.]

BARTON and E. R. H. JONES (*J.*, 1944, 659) postulated intramolecular hydrogen bonding of α- and β-boswellic acid and their esters to account for the deviation from standard values of observed molecular-rotation increments on esterification and oxidation of the hydroxyl group. Similar rotatory anomalies may be expected for other compounds which fulfil the electronic and steric requirements for chelation. Furthermore, since solvents can interfere with the intramolecular hydrogen bond of the solute by, for instance, acting as hydrogen donors or acceptors, the optical rotations of chelated compounds may be greatly influenced by solvent. An anomalous solvent effect has now been found for a group of steroid α-ketols, including 17α-hydroxy-20-keto-steroids which were recently shown (R. N. Jones, Humphries, Herling, and Dobriner, *J. Amer. Chem. Soc.*, 1952, **74**, 2820) to exhibit spectroscopic properties characteristic of chelated systems.



α-Boswellic acid.



β-Boswellic acid.

(Configuration of the carboxyl group in α-boswellic acid according to Vogel, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1951, **34**, 2321. The molecular fragments summarily represented by $C_{21}H_{34}$ are not identical for the two isomeric acids.)

Rotations of the compounds (I)—(VI) (see Table) were taken in chloroform, dioxan, acetone, and ethanol, *i.e.*, in the solvents most commonly used for steroids. The maximum changes of $[M]_D$ with solvent found for (I), (III), (IV) and (V) were 120°, 138°, 147°, and 145°, respectively, values which greatly exceed the normally observed maximum variation of *ca.* 40° (cf. Josephson, *Biochem. J.*, 1935, **29**, 1484; Plattner and Heusser, *Helv. Chim. Acta*, 1944, **27**, 748; Barton, *J.*, 1946, 1116; Barton and Brooks, *J.*, 1949, 2596). The

$[M]_D$ values of 3β : 5α-diacetoxycholestan-6-one (II) and 3α : 17α-diacetoxypregnane-11 : 20-dione (VI), in which the hydrogen bonding has been suppressed by acetylation of

Substance	$[M]_D$ in : $CHCl_3$	$C_4H_8O_2$	$COMe_2$	EtOH
3β-Acetoxy-5α-hydroxycholestan-6-one (I)	−263° (−257) [a]	−219°	−143°	−228° *
3β : 5α-Diacetoxycholestan-6-one (II)	− 70 (− 77) [a]	− 60	− 73	− 98
21-Acetoxy-17α-hydroxypregn-4-ene-3 : 11 : 20-trione (III)	+919 (+879) [b]	+885 (+852) [c]	+781 (+750) [b]	+898 *
3α : 17α-Dihydroxypregnane-11 : 20-dione (IV)	+132	+247	+235 (+239) [d]	+279
3α-Acetoxy-17α-hydroxypregnane-11 : 20-dione (V)	+222	+351	+309 (+327) [d]	+367
3α : 17α-Diacetoxypregnane-11 : 20-dione (VI)	+195 (+202) [e]	+220	+229	+201
$\Delta_1(5\alpha)$ for (I) ⟶ (II)	+193	+159	+ 70	+130
$\Delta_1(3\alpha)$ for (IV) ⟶ (V)	+ 90	+104	+ 74	+ 88
$\Delta_1(17\alpha)$ for (V) ⟶ (VI)	− 27	−131	− 80	−166

* With addition of chloroform. [a] Schenck, *Z. physiol. Chem.*, 1936, **243**, 119. [b] Kritchevsky, Garmaise; and Gallagher, *J. Amer. Chem. Soc.*, 1952, **74**, 483. [c] Turner, *ibid.*, 1953, **75**, 3604. [d] Sarett, *ibid.*, 1948, **70**, 1454. [e] Huang-Minlon, Wilson, Wendler, and Tishler, *ibid.*, 1952, **74**, 5394.

the ketolic hydroxyl group, vary with solvent by not more than 38° and 34°, respectively, *i.e.*, within the normal range. The rotation increments for (I) ⟶ (II), (IV) ⟶ (V), and (V) ⟶ (VI) [$\Delta_1(5\alpha)$, $\Delta_1(3\alpha)$, and $\Delta_1(17\alpha)$, respectively] can be calculated from rotations taken either in the same solvent or in different solvents. In the former case the range of variation with solvent is +70° to +193° for $\Delta_1(5\alpha)$, +74° to +104° for $\Delta_1(3\alpha)$ (standard value for 3α-hydroxy-5β-steroids is +83°; cf. Barton, *loc. cit.*), and −27° to −166° for $\Delta_1(17\alpha)$; in the latter case it is +45° to +203° for $\Delta_1(5\alpha)$, −55° to +255° for $\Delta_1(3\alpha)$, and +7° to −172° for $\Delta_1(17\alpha)$.

It is apparent that an anomalous solvent effect must be taken into consideration when applying the method of molecular-rotation differences to chelated compounds: when structural changes are studied at a site far removed from the chelated system, as in (IV) ⟶ (V), the $[M]_D$ increments must be computed from rotations taken in the same solvent, though it does not appear to matter which particular solvent is chosen. When the structural change involves the chelated system itself, as in (I) ⟶ (II) and (V) ⟶ (VI), it is essential to carry out all measurements in one solvent only. Failure to observe these precautions may lead to fortuitous and misleading results. An illustration is provided by Turner's recently attempted correlation (*loc. cit.*) of Δ_1 values for 17-hydroxypregnan-20-ones with their configuration at $C_{(17)}$. A compilation of relevant rotational data led Turner to conclude that acetylation of 17α-hydroxypregnan-20-ones gives rise to a large negative (>−100°) $[M]_D$ shift, while in the 17-epimeric series the shift is either small or positive. The cited examples include the increment of −125° for (V) ⟶ (VI) calculated from the rotations of (V) in acetone and of (VI) in chloroform; the same increment when computed from the rotations of (V) in chloroform and of (VI) in acetone (see Table) is +7°, *i.e.*, within the range allegedly characteristic for the 17α-pregnane series.

Experimental.—All specimens were dried in high vacuum for 3—6 hr. at 100—120°. M. p.s were determined on a Kofler stage. Rotations were taken at 16—18° in a 1-dm. micro-tube : for each compound the four successive sets of rotations refer respectively to solutions in chloroform, dioxan, acetone, and ethanol except where ethanol–chloroform is specified for the last.

3β-*Acetoxy*-5α-*hydroxycholestan*-6-*one* (I).—3β : 5α-Dihydroxycholestan-6-one was prepared from cholesterol *via* cholestane-3β : 5α : 6β-triol as described by Fieser and Rajagopalan (*J. Amer. Chem. Soc.*, 1949, **71**, 3938), and acetylated with acetic anhydride in pyridine at 18°. The crude acetate, recrystallised from acetone, had m. p. 231—233°, $[\alpha]_D$ −56°, −57·5°, −58°, −57·5°, −56·5° (*c*, 0·61, 0·92, 1·20, 1·96, 3·73), −47·5° (*c*, 0·88), −31° (*c*, 0·58), −49·5° (*c*, 0·81 in ethanol–chloroform; 80 : 20, v/v).

3β : 5α-*Diacetoxycholestan*-6-*one* (II).—This compound was prepared from the dihydroxy-ketone by Fieser and Rajagopalan's procedure (*loc. cit.*); crystallised from methanol, it had m. p. 171—173°, $[\alpha]_D$ −14° (*c*, 2·30), −12° (*c*, 1·67), −14·5° (*c*, 2·09), −19·5° (*c*, 1·68).

21-*Acetoxy*-17α-*hydroxypregn*-4-*ene*-3 : 11 : 20-*trione* (III).—The commercial preparation

(Merck & Co., Inc.) was recrystallised from ethanol and then had m. p. 238—240°, $[\alpha]_D$ +228° (*c*, 1·01), +220° (*c*, 0·94), +194° (*c*, 0·66), +223° (*c*, 0·52 in ethanol–chloroform; 80 : 20, v/v).

3α : 17α-*Dihydroxypregnane*-11 : 20-*dione* (IV).—A specimen supplied by Messrs. N. V. Organon (Oss) was recrystallised from acetone and then had m. p. 202—204°, $[\alpha]_D$ +38° (*c*, 1·56), +71° (*c*, 1·46), +67·5° (*c*, 1·06), +80° (*c*, 0·71).

3α-*Acetoxy*-17α-*hydroxypregnane*-11 : 20-*dione* (V).—This compound was obtained from the dihydroxy-ketone (IV) by acetylation with acetic anhydride in pyridine at 18° and had m. p. 203—204° after crystallisation from acetone–light petroleum, and $[\alpha]_D$ +57° (*c*, 1·40), +90° (*c*, 1·29), +79° (*c*, 1·05), +94° (*c*, 0·87).

3α : 17α-*Diacetoxypregnane*-11 : 20-*dione* (VI).—This compound was prepared by Turner's general procedure (*ibid.*, 1953, **75**, 3489) : 3α-acetoxy-17α-hydroxypregnane-11 : 20-dione (200 mg.) in glacial acetic acid (10 c.c.) and acetic anhydride (2 c.c.) was treated with toluene-*p*-sulphonic acid (200 mg.) and left overnight at 18°. The solution was diluted with water, and the product extracted with ether. Two crystallisations from ether–light petroleum furnished needles, m. p. 201—203°, $[\alpha]_D$ +45° (*c*, 1·13), +51° (*c*, 0·94), +53° (*c*, 1·06) +46·5° (*c*, 0·88) (Found : C, 69·3; H, 8·3. Calc. for $C_{25}H_{36}O_6$: C, 69·4; H, 8·4%).

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RHEUMATISM RESEARCH UNIT,
NETHER EDGE HOSPITAL, SHEFFIELD, 11. [*Received, November 4th*, 1953.]

The Reaction of Phenyl-lithium with Azobenzene.

By P. F. HOLT and B. P. HUGHES.

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WITTIG ("Newer Methods of Preparative Organic Chemistry," Interscience Publ., Inc., New York, 1948, p. 590, footnote) states that phenyl-lithium reacts with azobenzene to give triphenylhydrazine and hydrazobenzene but does not mention the conditions of the reaction. Gilman and Baillie (*J. Org. Chem.*, 1937, **2**, 84) using stated conditions could find no triphenylhydrazine but obtained diphenyl and hydrazobenzene. Beringer, Farr, and Sands (*J. Amer. Chem. Soc.*, 1953, **75**, 3984) confirmed the findings of Gilman and Baillie and used them to deduce the course of a reaction between phenyl-lithium and nitrous oxide which yielded lithium benzenediazoate, azobenzene and triphenylhydrazine. They argued that, since azobenzene and phenyl-lithium do not give triphenylhydrazine, the triphenylhydrazine produced in the last reaction must be formed by the action of phenyl-lithium on the lithium benzenediazoate rather than on the azobenzene.

We find that either triphenylhydrazine or hydrazobenzene may be formed.

If azobenzene (1 mol.) is stirred with phenyl-lithium (2 mol.) in ether for 40 hours as described by Gilman and Baillie, hydrazobenzene is formed. The following procedure gives triphenylhydrazine : Phenyl-lithium (1·6 g.) in dry ether (20 ml.) is added to azobenzene (3·6 g.) in dry ether (30 ml.) under nitrogen. The mixture is stirred for 5 minutes, then saturated ammonium chloride solution is added. The residue left on evaporation of the ethereal layer, when washed with light petroleum and recrystallised from ethanol, gives triphenylhydrazine, m. p. 141—142° (decomp.) (it yields *N*-phenylbenzidine, m. p. 136—137°, with hydrochloric acid in warm aqueous ethanol). The mother-liquor contains mainly hydrazobenzene.

The yield of triphenylhydrazine varies with the reaction temperature; it is 10% at 20° or 20% at 0°. The higher yield with decreased temperature conforms with the formation of triphenylhydrazine under the initial conditions which Beringer *et al.* used (−20° for 2 hr.). Gilman and Baillie considered that the hydrazobenzene was unlikely to be formed by the action of phenyl-lithium on the intermediate *N*-lithiotriphenylhydrazine because triphenylhydrazine does not react with phenylmagnesium bromide (Gilman and Adams, *J. Amer. Chem. Soc.*, 1926, **48**, 2004) which they considered analogous to phenyl-lithium. We find, moreover, that there is no reaction between triphenylhydrazine and phenyl-lithium under the conditions of the experiment. However this argument assumes that the reactivity of *N*-lithiotriphenylhydrazine is similar to that of triphenylhydrazine itself, which may be untrue.

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