the acidities of the respective carboxylic acids,[8] and probably also by the comparison of the rates of the alkaline hydrolysis of the carboxylic esters.[9]

It is noteworthy that the principal absorption bands of the four chalcone analogs, in which the 2-thienyl and 2-furyl groups replace both of the phenyl groups, are located within a very narrow range. This would seem to imply the absence of any special resonance contributions by the 2-thienyl group in which the sulfur atom has an expanded valence shell.

Experimental

All of the heterocyclic chalcone analogs were prepared according to the directions of Weygand and Strobelt.[10] The absorption spectra were determined in 95% ethanol using a Beckman DU spectrophotometer. The spectral characteristics of the compounds discussed here are summarized in Table I, and the absorption curves of all of the compounds are reproduced in Figs. 1 and 2.

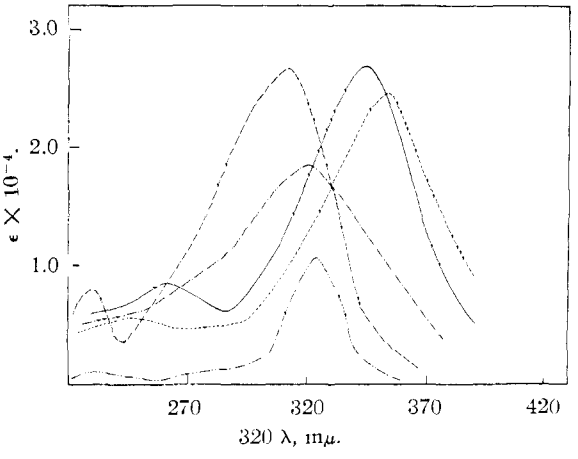

Fig. 1.—Ultraviolet absorption spectra of compounds listed in Table I: — —, no. 1; ———, no. 3; —·—·, no. 4; —·—·—·, no. 5; ·····, no. 7.

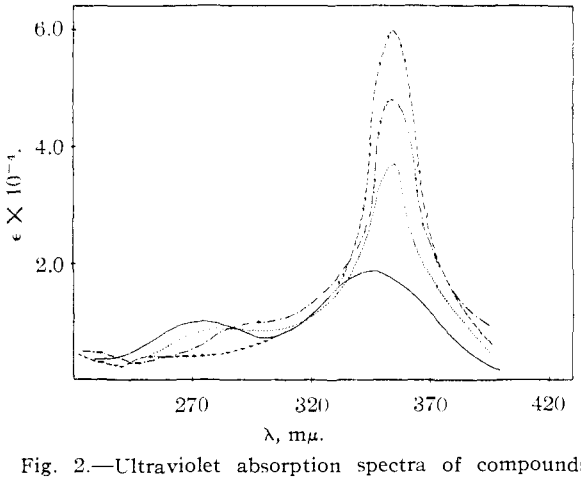

Fig. 2.—Ultraviolet absorption spectra of compounds listed in Table I: ——, no. 2; . . . . . , no. 6; — — —, no. 8; —·—·—, no. 9.

(8) W. Catlin [*Iowa State Coll. J. Sci.*, **10**, 65 (1935)] reports the ionization constants of 2-furoic and 2-thiophenecarboxylic acids as 75×10^{-5} and 34×10^{-5}, respectively. Both of these acids are thus considerably stronger than benzoic acid (6.3×10^{-5}).

(9) See ref. 6 for the discussion of these results.

(10) C. Weygand and F. Strobelt, *Ber.*, **68B**, 1839 (1935).

Acknowledgment.—We wish to thank the Research Corporation for financial support of this work.

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A Preparation of Progesterone-4-C^{14}

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RECEIVED SEPTEMBER 18, 1953

This investigation was undertaken to fulfill the need for substantial quantities of ring-labeled progesterone-C^{14} required as a starting material for biological and chemical conversion to radioactive corticosteroids.[1] By including radiocarbon in the 4-position of the steroid nucleus, it was hoped that the label would persist in the metabolic fragments as indicated by studies conducted with cholesterol-4-C^{14}.[2]

Three procedures have been published concerning the inclusion of radiocarbon in the α,β-unsaturated 3-ketone grouping of several steroids, the phenyl acetate method,[3-5] the Reformatsky reaction[3,6] and the Grignard reaction.[7-9] In view of the reported superior yields and adaptability to larger scale synthesis, the latter procedure was used to prepare methyl 3-keto-Δ^4-etienate (Ia). Experience in these laboratories has shown that this substance is converted in excellent yield to desoxycorticosterone acetate and progesterone by modifications of published procedures.[6,10]

When methyl 3-keto-Δ^4-etienate (Ia) was ozonized[3] in 3-g. lots, at least 90% was transformed to acidic material from which a 70–75% theoretical yield of keto acid IIa[11] was obtained. Periodate oxidation[3] of the neutral residue gave small additional amounts of IIa. The separation of neutrals from acidics was readily effected using sodium carbonate. Dilute sodium hydroxide caused concurrent saponification of the labile 17-carbomethoxy group of IIa.[12] In contradistinction, methyl ester

(1) Progesterone-4-C^{14} was the key intermediate in the recently completed partial synthesis of cortisone-4-C^{14} acetate—a project arranged under contract with the Endocrinology Study Section of the National Institutes of Health, U. S. Public Health Service. The project was directed by a committee of the Study Section consisting of Dr. C. Huggins, chairman, Dr. S. R. Hall, executive secretary, and Drs. T. F. Gallagher, Sloan-Kettering Institute, M. Tishler of Merck & Co., Inc., and G. Pincus of the Worcester Foundation, and by Dr. A. D. Odell, formerly of Frosst & Co. but now with Syntex, S. A. Progesterone-4-C^{14} was converted to pregnane-3,11,20-trione-4-C^{14} by Drs. H. G. Kolloff and R. H. Levin of the Upjohn Co., Kalamazoo, Mich., and returned to us for completion of the synthesis of cortisone acetate by known methods; *cf.* T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, THIS JOURNAL, **74**, 483 (1952).

(2) I. L. Chaikoff, M. D. Siperstein, W. G. Dauben, H. L. Bradlow, J. F. Eastham, G. M. Tomkins, J. R. Meier, R. W. Chen, S. Hotta and P. A. Srere, *J. Biol. Chem.*, **194**, 413 (1952).

(3) R. B. Turner, THIS JOURNAL, **72**, 579 (1950).

(4) J. Ashmore, W. H. Elliott, E. A. Doisy, Jr., and E. A. Doisy, *J. Biol. Chem.*, **200**, 661 (1953).

(5) M. Gut, *Helv. Chim. Acta*, **36**, 906 (1953).

(6) R. D. H. Heard and P. Ziegler, THIS JOURNAL, **72**, 4328 (1950).

(7) B. Belleau, Thesis, McGill University, 1950.

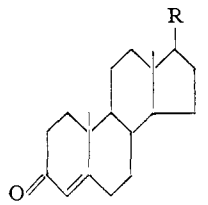
(8) (a) G. I. Fujimoto, THIS JOURNAL, **73**, 1856 (1951); (b) G. I. Fujimoto and J. Prager, *ibid.*, **75**, 3259 (1953).

(9) R. D. H. Heard and P. Ziegler, *ibid.*, **73**, 4036 (1951).

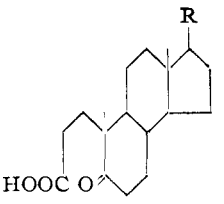
(10) A. L. Wilds and C. H. Shunk, *ibid.*, **70**, 2427 (1948).

(11) T. Reichstein and H. G. Fuchs, *Helv. Chim. Acta*, **23**, 676 (1940).

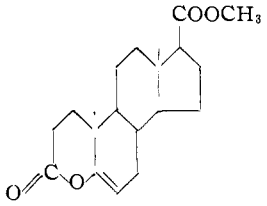
(12) J. R. Jamieson, unpublished data from these laboratories.



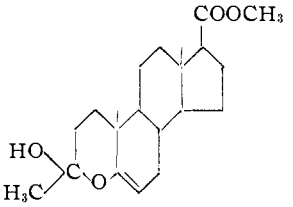
Ia, R = $COOCH_3$
b, R = COOH
c, R = $COCH_3$



IIa, R = $COOCH_3$
b, R = COOH



III



IV

Ia is remarkably stable to the action of these bases and this fact was utilized at a later stage for the recovery of pure labeled methyl ester Ia from a large amount of extraneous neutral product. The formation of enol lactone III from IIa proceeded smoothly in refluxing acetic anhydride containing 9% anhydrous sodium acetate.

In a large series of Grignard reactions conducted at temperatures ranging from 20 to −75°, it was observed that the order of addition of Grignard reagent and enol lactone III was of no apparent importance. An evaluation of the yield data indicated that optimum conditions for radiosynthetic work would entail addition of 1.0 to 1.1 equivalents of methylmagnesium iodide to a solution of III at room temperature. Separation of the carbinol IV from unchanged enol lactone III was possible by hydrolysis of the collected Grignard complex. This technique, however, was discarded because precipitation of this complex was incomplete. Although IV resulting from the hydrolysis of the reaction mixture could be crystallized (m.p. 172.5–174.5°) with prohibitive losses, it was preferable to subject the crude IV directly to alkaline rearrangement. Prolonged refluxing with methanolic sodium hydroxide gave excellent conversion to acidic material from which only the impure acid Ib was obtained. On the other hand, milder treatment allowed the isolation of the methyl ester of Ib. Thus when crude IV was dissolved in the same medium but kept at 20° for 1.5 hours, the desired rearrangement occurred giving the methyl ester Ia and a readily separated acidic fraction. Spectroscopic examination of the latter following crystallization indicated the presence of a small amount of Ib, whereas the anticipated products were those arising from the action of alkali on unchanged enol lactone III, *viz.*, IIa and IIb. Furthermore, methyl ester Ia suffers no saponification under these conditions. Compound Ib is believed to have arisen by saponification of some IV prior to its rearrangement. It was recovered as the methyl ester Ia by esterifying the total acidic fraction with diazomethane and re-submitting the neutral mixture to the conditions of the mild alkaline rearrangement. The yield of methyl ester Ia based on methyl iodide was 55–60%.

Saponification of Ia to Ib followed by an Arndt–Eistert extension[13] and treatment with hydriodic acid[6] gave progesterone (Ic). Several inactive trials indicated that an over-all conversion of 15–20%, based on methyl iodide, could be anticipated. When freshly prepared methyl iodide-C^{14} was used, progesterone-4-C^{14} was obtained in 30–37% yield.

Acknowledgment.—The authors wish to express their thanks to Mr. R. W. Cumming for technical assistance and in particular for the large scale preparation of the required starting materials. We are indebted to Dr. J. R. Jamieson who designed and constructed the specialized glass apparatus.

Experimental[14]

Starting Material.—Methyl 3-keto-Δ^4-etienate (Ia) was ozonized[3] and the resulting keto acid IIa[11] lactonized to enol lactone III, m.p. 168–170°, $[\alpha]^{24}D$ −34° ($CHCl_3$).[15]

Grignard Reaction with Enol Lactone III.—Into a flask containing 654 mg. (26.9 mmoles) of activated magnesium turnings was distilled *in vacuo* and frozen 3.4 g. (23.9 mmoles) of methyl iodide-C^{14} containing 72 mc. of C^{14}.[16] Absolute ether (25 ml.) was added and the contents of the flask allowed to warm to room temperature. When the initial vigorous reaction had subsided, the dark-colored mixture was refluxed for 30 min. and then diluted with 75 ml. of absolute ether.

The Grignard reagent was added dropwise over a period of 70 min. at room temperature to a stirred solution of 7.944 g. (23.9 mmoles) of enol lactone III in 70 ml. of benzene and 120 ml. of ether. The thick suspension was stirred an additional 20 min. After dilution of the reaction product with 6.0 ml. of 37% hydrochloric acid in 175 ml. of saturated saline solution, the precipitate dissolved and was replaced by a light deposit of glistening material. The aqueous phase was transferred to a separatory funnel and repeatedly extracted with ether. All organic phases were combined and filtered from the over-reacted material.[17] The filtrate was washed successively with saturated saline, 5% sodium bicarbonate, 5% sodium thiosulfate and finally with water to neutrality. Removal of the solvent under reduced pressure gave an oily residue of labeled carbinol IV.

From two Grignard reactions (72 and 76 mc. of methyl iodide) there resulted 1.002 g. of over-reacted material, m.p. 215–230°, which was not further investigated.

Methyl 3-Keto-Δ^4-etienate-4-C^{14} (Ia-4-C^{14}).—The crude labeled IV (*ca.* 8 g.) was rearranged by stirring at room temperature for 90 min. in 400 ml. of methanol containing 33 ml. of aqueous 6 *N* sodium hydroxide. The solution was concentrated to a small volume *in vacuo* at less than 40°, the residue diluted with saturated saline and extracted several times with ether–chloroform (3:1). The combined extracts were freed of a small amount of insoluble matter and washed to neutrality. Evaporation afforded 5.5 g. of a green resin from which there was obtained by crystallization 4.257 g. of methyl ester-4-C^{14}, m.p. 132–135.5°. Chromatography of the liquors over alumina (Alcoa F-20) gave an additional 245 mg. of Ia-4-C^{14}. The total yield based on methyl iodide-C^{14} was 57%.

In a second run using methyl iodide-C^{14} (25.8 mmoles, 76 mc.), the yield of Ia-4-C^{14} was 57.8%.

The aqueous alkaline phase from the above work-up was acidified to *p*H 1 and extracted with ether giving 2.6 g. of white foamy resinous acidic material. Crystallization from ether gave 0.58 g., m.p. 190–205°, and this was combined with 1.194 g. derived from 2.4 g. of similar acidics recovered from the second synthesis. A solution of these crystalline acidics in 30 ml. of chloroform was esterified with diazo-

(13) W. E. Bachmann and W. S. Struve, "Organic Reactions," John Wiley & Sons, Inc., New York, N. Y., 1942, Ch. 2, pp. 38–62.

(14) All melting points were taken on a Fisher–Johns apparatus and viewed with a microscope.

(15) This substance was identical with a sample previously prepared by Dr. P. Ziegler at McGill University, unpublished data.

(16) Prepared by Tracerlab, Inc., Boston, Mass., from C^{14} supplied by the U. S. Atomic Energy Commission.

(17) *Cf.* R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W. M. McLamore, THIS JOURNAL, **74**, 4223 (1952).

methane. Concentration to dryness and crystallization from ether furnished 75 mg. of Ia-4-C^{14}. The remaining acidic residues were esterified similarly and added to a pool consisting of all residual fractions remaining after isolation of Ia-4-C^{14}. Treatment with an equal weight of sodium hydroxide (aqueous 6 *N*) in 50 parts of methanol for 1.5 hours at room temperature gave a neutral fraction (1.16 g.) from which there separated an additional 0.323 g. of labeled ester Ia, m.p. 126–132°.

The total yield of radioactive Ia based on 148 mc. of methyl iodide-C^{11} was 9.831 g., 29.75 mmoles, 59.8%.

Progesterone-4-C^{14} (Ic-4-C^{14}).—A solution of 5.4026 g. of Ia-4-C^{14} in 90 ml. of methanol and 13.5 ml. of aqueous 6 *N* potassium hydroxide was refluxed for 6 hours.[17] After the usual work-up, there resulted 4.127 g. of unpurified Ib-4-C^{14}, m.p. 225–230°, 95.7%. This material was diluted with inactive acid Ib[18] to a total weight of 8.422 g. (calculated activity, 1.47 mc. per mmole).

The sodium salt of Ib-4-C^{14} was prepared by a modification of the method of Wilds and Shunk[10] employing 0.95 equivalent of methanolic sodium hydroxide. The acid chloride, prepared in ether solution[19] by means of oxalyl chloride, was treated with excess diazomethane[13] to afford 21-diazoprogesterone-4-C^{14}, 4.39 g., m.p. 178–180° dec., and 3.76 g., m.p. 174–176° dec.[10] The yield of 21-diazoprogesterone-4-C^{14} was 90.1% based on Ib-4-C^{14}. A second Arndt–Eistert reaction using 9.711 g. of Ib-4-C^{14} (calculated activity, 1.47 mc. per mmole.), gave an 89.1% conversion to 21-diazoprogesterone-4-C^{14}.

Treatment[6] of the above diazoketone with 47% hydriodic acid gave an 87% yield of crude progesterone-4-C^{14} (Ic-4-C^{14}), m.p. 120–128°. Recrystallization from ether (activated charcoal) afforded 11.814 g., m.p. 127–131°, $[\alpha]^{21}D$ 203 ± 3° ($CHCl_3$), 37.2% based on methyl iodide-C^{14}. The specific activity per mmole. was found[20] to be 1.52 mc.; calculated activity 1.47 mc. A portion (5.459 g.) of this material was freed from a final trace of color by chromatography over untreated alumina (Alcoa F-20) to give an 81% recovery of pure progesterone-4-C^{14} (Ic-4-C^{14}), m.p. 132–133.5°, $[\alpha]^{21}D$ 202 ± 2° ($CHCl_3$), $\lambda_{max}^{ethanol}$ 240 mμ (ε 16680).

(18) The variability of the reported constants for this substance has prompted us to record those of a purified sample. Following several crystallizations from chloroform and then ethyl acetate, the data were: m.p. 253–257°, $[\alpha]^{25}D$ 165° ($CHCl_3$) and $\lambda_{max}^{ethanol}$ 238.5–240 mμ (ε 17750).

(19) C. H. Gleason, unpublished data from these laboratories.

(20) R. D. H. Heard, L. Thompson and C. Yates, *Proc. Can. Physiol. Soc.*, 23 (1951).

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Platinum-catalyzed Exchange of Hydrogen Isotopes with Bile Acids

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RECEIVED OCTOBER 14, 1953

In the application of stable hydrogen isotope tracer techniques to bile acids, it is necessary to introduce deuterium into positions where it will remain stably bound under the conditions employed in the tracer experiments. We have found a method for obtaining as many as eight stably bound atoms of deuterium per molecule of either desoxycholic acid or cholic acid. This constitutes a considerable improvement over the results reported thus far with platinum-catalyzed hydrogen isotope exchange on steroids in deuterioacetic acid.[1–5] Our departure from the procedure of previous workers is in the use of deuterium oxide and alkali as the reaction medium for the platinum-catalyzed exchange.

(1) K. Bloch and D. Rittenberg, *J. Biol. Chem.*, **149**, 505 (1943).

(2) H. S. Anker and K. Bloch, THIS JOURNAL, **66**, 1752 (1944).

(3) D. K. Fukushima and T. F. Gallagher, *J. Biol. Chem.*, **198**, 861 (1952).

(4) D. K. Fukushima and T. F. Gallagher, *ibid.*, **198**, 871 (1952).

(5) J. Bell and S. J. Thomson, *J. Chem. Soc.*, 572 (1952).

Experimental

Desoxycholic acid was purified by refluxing with carbon tetrachloride (1 liter per 20 g. of acid), filtering while hot, drying *in vacuo* at 25°, recrystallizing from acetone and drying *in vacuo* at 155°.

The reaction vessel used in the deuterization consisted of a narrow-necked 100-ml. Pyrex cylinder which fitted by means of a ground glass joint into a vapor jacket equipped with side arms for boiler and condenser. Both were flat-bottomed to permit magnetic stirring of the reaction mixture in the sealed cylinder. Methyl cellosolve was used to maintain the temperature at 124°.

Platinum catalyst was prepared by reduction of platinum dioxide with hydrogen in water suspension, followed by several washes with deuterium oxide to remove the water and to replace the hydrogen sorbed on the catalyst surface with deuterium.

A typical reaction mixture consisted of 10 g. of pure desoxycholic acid (m.p. 174–175°), 50 ml. of deuterium oxide (99.8%) containing 2 g. (2.05 equiv.) of sodium hydroxide and the platinum obtained by reducing 1.5 g. of platinum dioxide as described above. After the introduction of a glass-covered stirring bar, the reaction cylinder was flushed with nitrogen and sealed.

The reaction was allowed to proceed at 124° with stirring for seven days. After removal of the catalyst by centrifugation, the solution was diluted with 125 ml. of distilled water and acidified with hydrochloric acid to precipitate the deuterized desoxycholic acid. The product was filtered off, washed with water until chloride-free, dried *in vacuo* at 25° and crystallized twice from acetone, using 4 g. of Darco G60 the first time. The crystals were dried *in vacuo* at 155°; yield 3.3 g. of deuteriodesoxycholic acid, m.p. 173–174°, atom per cent. deuterium 18.4. The deuterium content was *unchanged* by refluxing one-half hour in methanol containing 5% potassium hydroxide. Repeat experiments gave materials containing 17.8, 13.7 and 20.0 atom per cent. deuterium, respectively. Further experiments with different amounts of catalyst and for extended periods of time have provided no unequivocal evidence as to the causes for this variation in extent of deuterium exchange. Fluctuations in temperature, poisoning of the catalyst by degradation products or by extraneous impurities may all be factors.

Cholic acid was submitted to deuterium exchange in a similar manner. However, cholic acid was found to differ markedly from desoxycholic acid in its susceptibility to degradation. Whereas only small amounts of by-products were formed in the case of desoxycholic acid, the side-reactions in the case of cholic acid consumed about half the starting material. This is what might be expected in view of the well-known lability at the 7-hydroxyl position in this substance. A more elaborate purification process was, therefore, required. Based upon preliminary experiments which included a chromatographic study of the crude reaction product, an isolation procedure was devised which is illustrated by the following example. Thirty grams of cholic acid was treated in a stainless steel autoclave with 180 ml. of deuterium oxide containing 6.5 g. (2.3 equiv.) of sodium hydroxide. Seven grams of a 10–13% platinum-on-charcoal catalyst was added. The reaction was carried out under nitrogen for 2 days at 115° with stirring. After cooling, the catalyst was removed by filtration. The filtrate was acidified with 75 ml. of 2.5 *N* hydrochloric acid and 10 g. of sodium sulfate was added. This solution was extracted three times with an equal volume of a mixture containing 1 part isopropyl alcohol to 2 parts ethyl ether. The combined non-aqueous phase was washed and dried over anhydrous sodium sulfate, then evaporated to dryness, with occasional small additions of acetone to facilitate removal of the isopropyl alcohol. The residue was a brown gummy mass containing 20.0 atom per cent. deuterium. This residue was taken up in acetone and treated with an excess (about 7 g.) of fresh diazomethane in ether. After the esterification was complete, excess ether was removed and enough acetone was added to make the total volume equal one liter. This acetone solution was charged on to a chromatographic column containing 400 g. of dry alumina, acid-washed to neutrality. The charged column was then washed