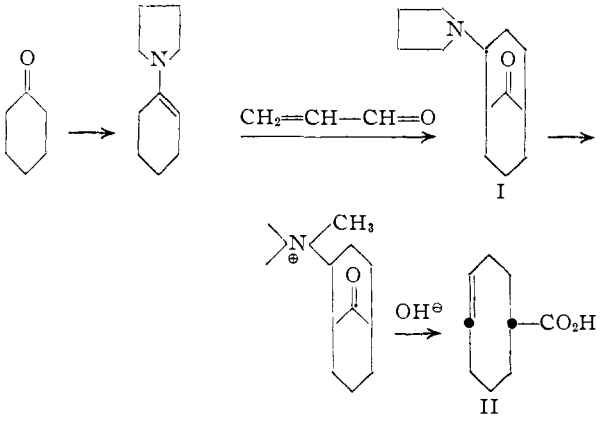
Reaction of the pyrrolidine enamine of cyclohexanone with one equivalent of acrolein in dioxane, initially at 0°, gave 75% of I, b.p. 125–127° (0.5 mm.); picrate, m.p. 171–172° (found: C, 52.42; H, 5.51). I could be reduced in 72% yield by the Wolff–Kishner method to the corresponding desoxo compound, b.p. 145–147° (15 mm.); *picrate*, m.p. 147–148° (found: C, 54.33: H, 6.11).



The methiodide of I was transformed, on heating with aqueous base, to 4-cycloöctenecarboxylic acid (II), b.p. 118–120° (0.4 mm.); amide, m.p. 201–202° (found: C, 69.89; H, 9.63). Catalytic reduction of II gave the known cycloöctanecarboxylic acid.[1]

In a similar manner, the pyrrolidine enamine of cyclopentanone and acrolein led after one hour at room temperature to 55% of bicyclic aminoketone, b.p. 110–115° (0.5 mm.): *picrate*, m.p. 180–181° (found: C, 51.31; H, 5.39). This gave, *via* its methiodide, 4-cycloheptenecarboxylic acid, m.p. 65–67° (found: C, 68.38; H, 8.70). It is presumed that the new reaction will be applicable to substituted cyclic ketones and also to substituted acroleins. This point will have to be established.

(1) This was compared as its amide by mixed melting point with a sample kindly supplied by Prof. A. C. Cope: A. C. Cope and H. O. Van Orden, THIS JOURNAL, **74**, 175 (1952).

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RECEIVED AUGUST 31, 1956

LOCATION OF THE SIXTH HYDROXYL GROUP IN OUABAGENIN

Sir:

On the basis of evidence summarized recently[1] the positions (1, 3, 5, 14, and 19) of five of the six hydroxyl groups of ouabagenin may be regarded as firmly established. The location of the sixth (secondary) hydroxyl function has remained unsettled, although certain observations of Tschesche and Snatzke[2] suggest that this group is attached at C.11 (α) in agreement with a tentative proposal advanced some years ago by Fieser and Newman.[3]

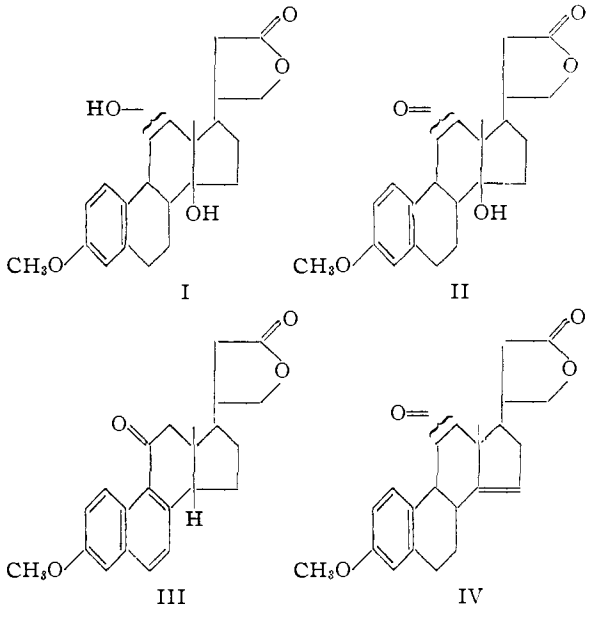
(1) K. Florey and M. Ehrenstein, *J. Org. Chem.*, **19**, 1174 (1954); R. P. A. Sneeden and R. B. Turner, THIS JOURNAL, **77**, 130 (1955); Ch. Tamm, *Helv. Chim. Acta*, **38**, 147 (1955).

(2) R. Tschesche and G. Snatzke, *Ber.*, **88**, 1558 (1955).

(3) L. F. Fieser and M. S. Newman, *J. Biol. Chem.*, **114**, 705 (1936); see also C. Mannich and G. Siewert, *Ber.*, **75**, 737, 750 (1942).

We have now obtained definitive evidence in support of the C.11 assignment.

Oxidation of the diol I, obtained in the earlier investigation,[1] with the chromium trioxide–pyridine complex furnishes a ketol (II), m.p. 189–191°, $[\alpha]_D$ +182° (*c* 1.34, acetone), $\lambda_{max.}$ 2.80, 5.62, 5.80 μ, (*Anal.* Calcd. for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 71.60; H, 7.54). The ultraviolet spectrum of II ($\lambda_{max.}$ 276, 284 mμ, ϵ 1626, 1522) shows only anisole absorption, and the absence of a conjugated carbonyl system in this substance is



further established by the position (5.80 μ) of the ketonic absorption band in the infrared. The suggestion of Djerassi and Ehrlich[4] that the hydroxyl group in question may occupy the 6-position in ouabagenin is thereby excluded.

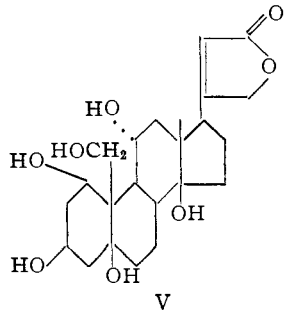
Palladium dehydrogenation of II at 260° proceeds with concomitant loss of the 14-hydroxyl group, and yields a product (III), m.p. 260.5–262.5°, $[\alpha]_D$ +159° (*c* 0.97, chloroform), (*Anal.* Calcd. for $C_{23}H_{24}O_4$: C, 75.80; H, 6.64. Found: C, 75.91; H, 6.91), the ultraviolet absorption of which ($\lambda_{max.}$ 220, 246, 312, 348 mμ, log ϵ 4.75, 4.56, 3.94, 3.63) is virtually indistinguishable from that of *cis*-3-methoxy-11-ketoequilinane ($\lambda_{max.}$ 220, 246, 312, 348 mμ, log ϵ 4.79, 4.60, 3.93, 3.64), synthesized in an unambiguous manner by Eglinton, Nevenzel, Scott and Newman.[5] Compound III can also be obtained by dehydrogenation of IV, m.p. 236–242° (dec.), $[\alpha]_D$ +270° (*c* 1.01, chloroform), $\lambda_{max.}$ 276, 284 mμ, ϵ 1559, 1471, (*Anal.* Calcd. for $C_{23}H_{26}O_4$: C, 75.38; H, 7.15. Found: C, 75.15; H, 7.24), which was prepared from I by acetylation and dehydration,[1] hydrolysis and oxidation with the chromium trioxide–pyridine complex.

There can be no doubt that the chromophoric systems present in III and in *cis*-3-methoxy-11-ketoequilinane are the same. This evidence estab-

(4) C. Djerassi and R. Ehrlich, *J. Org. Chem.*, **19**, 1351 (1954).

(5) G. Eglinton, J. C. Nevenzel, A. I. Scott and M. S. Newman, *Chem. and Ind.*, 686 (1953); THIS JOURNAL, **78**, 2331 (1956). We are indebted to Professor Newman for samples of *cis*- and *trans*-3-methoxy-11-ketoequilinane for direct spectral comparison with III.

lishes C.11 as the position of the hydroxyl and keto



V

functions in I, II, and IV and, coupled with Tschesche's stereochemical results, permits assignment of structure V to ouabagenin.

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RECEIVED AUGUST 20, 1956

A CRUCIAL TEST OF TRANSMETHYLATION *in Vivo* BY INTRAMOLECULAR ISOTOPIC LABELING

Sir:

In an earlier study of transmethylation[1] in the rat reported from this laboratory, use was made of methionine doubly labeled in the methyl group by mixing molecules labeled separately with deuterium and C^{14}, a type of labeling referred to as *intermolecular*. It was found in this experiment that the ratio of deuterium to C^{14} in the methyl groups of tissue choline and creatine was the same as that ratio in the labeled methionine administered in the diet. This result was taken as a demonstration that the methyl group of methionine is transferred as a unit in the biosynthesis of choline and creatine.

In later experiments[2,3] on the neogenesis of methyl groups in rats from methanol and formate triply labeled intermolecularly with deuterium, tritium and C^{14}, a hydrogen isotope effect was shown to occur in the cleavage of carbon–hydrogen bonds resulting in the retention of the heavier isotopes of hydrogen. Thus the disproportionation of $D:C^{14}$ values from precursors to methyl groups was due not only to metabolic changes but also to a hydrogen isotope effect. A similar isotope effect was found in the oxidation of the methyl group of methionine to carbon dioxide when the singly labeled methyl group, $C^{14}H_3-$, was compared to the doubly labeled methyl group, $C^{14}D_3-$, containing both deuterium and C^{14} bonded together in the same molecule, a type of double labeling referred to as *intramolecular*.[4]

It occurred to us that, in view of the extensive hydrogen isotope effects observed with intermolecularly multiply labeled compounds, our earlier transmethylation study[1] may have involved a fortuitous compensation of a metabolic loss of hydrogen from the methyl carbon of methionine by a retention of deuterium by this carbon through a hydrogen isotope effect, and thus the methyl group may only have appeared to be transferred as a unit.[5] It therefore became necessary to restudy transmethylation from methionine in such a way that isotope effects would be avoided. It has been shown in this laboratory[6] that it is possible to eliminate the effect of hydrogen isotope selection in the metabolism of formate by intramolecular double labeling with deuterium and C^{14}.

Thus, for the transmethylation study, L-methionine containing D and C^{14} in the same methyl group was prepared[4] and diluted so that the D content was about the same as in the earlier investigation.[1] The labeled methionine was administered in an amino acid diet to three male white rats, each of about 160 g., according to the plan used in the previous study,[1] with the exceptions that vitamin B_{12} at a level of 15 micrograms per cent. was added to the vitamin supplement and that the administration was for three days. The food intake was limited to 10 g. per day. At the end of the third day, the animals were killed by ether anesthesia, the livers removed, and choline and creatine were isolated from the remainder of the carcasses.[7] Choline was analyzed as the chloroplatinate for deuterium and C^{14}. Choline was then degraded to trimethylamine which was analyzed as the chloroplatinate for deuterium and C^{14}. Creatine, isolated as creatinine potassium picrate, was degraded without prior isotopic analysis to methylamine which was analyzed as the chloroplatinate for deuterium and C^{14}.

Table I lists the analytical results which indicate (a) that under the conditions of this experiment

TABLE I

ISOTOPE CONTENT OF THE METHYL GROUPS OF ADMINISTERED METHIONINE AND ISOLATED COMPOUNDS

Rat	Compound[a]	Deuterium, atom % excess	C^{14}, cpm/meq. methyl $\times 10^{-6}$	$D:C^{14}$ $\times 10^6$	$D:C^{14}$ isol. methyl / $D:C^{14}$ methionine methyl
	Methionine	64.5	70.1	9.20	
R-2	Choline[b]	5.20	5.56	9.34	1.01
	TMA[c]	5.18	5.70	9.08	0.99
	MMA[d]	3.60	4.80	8.83	0.96
R-5	Choline[b]	5.34	5.81	9.19	1.00
	TMA[c]	5.38	5.82	9.24	1.00
	MMA[d]	4.49	5.03	8.93	0.97
R-7	Choline[b]	5.21	5.87	8.88	0.97
	TMA[c]	5.27	5.87	8.98	0.98
	MMA[d]	4.89	5.45	8.97	0.98

[a] With the exception of methionine, all compounds were analyzed as the chloroplatinates. [b] It was assumed in the analysis of choline that the total isotope content resided in the methyl groups. [c] TMA = trimethylamine derived from choline. [d] MMA = monomethylamine derived from creatine.

(1) E. B. Keller, J. R. Rachele and V. du Vigneaud, *J. Biol. Chem.*, **177**, 733 (1947).

(2) J. R. Rachele, E. J. Kuchinskas, J. E. Knoll and M. L. Eidinoff, THIS JOURNAL, **76**, 4342 (1954).

(3) J. R. Rachele, E. J. Kuchinskas, J. E. Knoll and M. L. Eidinoff, presented at a meeting of the New York Section of the American Chemical Society, March 16, 1956.

(4) J. R. Rachele, E. J. Kuchinskas, F. H. Kratzer and V. du Vigneaud, *J. Biol. Chem.*, **215**, 593 (1955).

(5) We wish to acknowledge a personal communication from Dr. H. R. V. Arnstein of the National Institute for Medical Research, London, expressing a similar opinion with respect to hydrogen isotope effects on transmethylation.

(6) J. R. Rachele and H. Aebi, *Federation Proc.*, **15**, 333 (1956).

(7) V. du Vigneaud, C. Ressler, J. R. Rachele, J. A. Reyniers and T. D. Luckey, *J. Nutrition*, **45**, 361 (1951).