effects in solvolysis reactions[1,2] and (2) the effect of alkyl substitution on hyperconjugation.[3-8]

I-1,1,1-d_3 was made via the corresponding alcohol from 4,4-dimethyl-2-pentanone and CD_3MgI. I-3,3-d_2 was made *via* the following sequence: ethyl pivalate, neopentyl-α-d_2 alcohol, neopentyl-α-d_2 iodide,[9] *t*-butylacetic-α-d_2 acid, ethyl *t*-butylacetate-α-d_2 and 2,4,4-trimethyl-2-pentanol-3,3-d_2. The deuterated chlorides were about ninety per cent. isotopically pure. The solvolysis rate constants were measured conductimetrically in "80%" aqueous ethanol at 25.00°.

SOLVOLYSIS RATE CONSTANTS

Compound	k_1 (10^{-4} sec.$^{-1}$)	Reproducibility (3 or 4 runs)
2,4,4-Trimethyl-2-chloropentane (I)	2.212	±0.015
I-1,1,1-d_3	1.580	±0.010
I-3,3-d_2	2.04	±0.03

ISOTOPE EFFECT OF DEUTERATION, SOLVOLYSIS IN "80%" ALCOHOL AT 25°

Group deuterated	Compound	k_H/k_D
CH_3-	*t*-Amyl chloride[1b]	1.34
CH_3-	I	1.40
-CH_2-	*t*-Amyl chloride[1b]	1.40
-CH_2-	I	1.08

The important fact to be noted is that the isotope rate effect of α-deuteration is so much smaller for the neopentyl than for the ethyl or methyl groups. Since these isotope rate effects arise from a difference in zero-point energy between the initial and transition states,[1,2] the conclusion that the α-C–H bonds of the neopentyl group are not weakened in the reaction process nearly as much as those of the methyl or ethyl groups seems certain. It follows that the electron loss from the α-C–H bonds of the neopentyl group is also relatively much less. This must be correlated with the fact that the neopentyl group is known to be a poorer electron releaser than the ethyl group in many reactions.[3,4,7]

If this parallelism between the low isotope rate effect of deuteration of the neopentyl group and its low electron releasing ability is accepted then the following explanations which have been offered for the latter phenomena can be discarded as not predicting the correct trends in the isotope rate effects: steric shielding of the reaction center,[10] second order hyperconjugation,[4] inductive effect on hyperconjugation,[7] and steric inhibition of bond contraction.[8] The preferred explanation which apparently best fits the data involves the following points: (1) *hyperconjugative loss of electron density from the α-C–H bonds to the reaction center is necessary for the isotope rate effect* and (2) *a condition for maximum hyperconjugative loss is that the C–H bonds have the proper orientation, nearly parallel, to the electron deficient adjacent orbital*[6] and (3) *that the end methyl groups of the neopentyl group sterically hinder the approach of the methylene group to the proper orientation for maximum hyperconjugation.*

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STEROID 21-HYDROXYLATION BY ADRENAL MICROSOMES AND REDUCED TRIPHOSPHOPYRIDINE NUCLEOTIDE[1]

Sir:

Biological reactions involving liver microsomes include protein[2] and cholesterol[3] syntheses and drug oxidation.[4] The enzymatic step for the 21-hydroxylation of 17α-hydroxyprogesterone has now been shown to occur in beef adrenal microsomes.

Plager and Samuels[5] and Hayano and Dorfman[6] have previously reported ATP and DPN dependent steroid 21-hydroxylation in beef adrenal extracts.

In the present study, beef adrenal fractions were prepared by differential centrifugation as described by Schneider and Hogeboom.[7] A 1:1 homogenate was prepared in 0.25 *M* sucrose, 0.1 *M* phosphate buffer at *p*H 6.8 and 0.04 *M* niacinamide. The microsomal fractions were obtained between 20,000*g* and 105,000*g*, washed and recentrifuged. The 105,000*g* supernatant was further purified by discarding protein precipitated at *p*H 5. Steroids were dissolved in propylene glycol and added to tissue fractions which were then incubated for one hour at 37° with air as the usual gas phase. Incubation mixtures were extracted with methylene chloride, evaporated and the residues partitioned between 95% methanol and hexane. The aqueous methanol was evaporated and the enzymatic conversion of 17α-hydroxyprogesterone to 17,21-dihydroxyprogesterone (Reichstein's Substance S) was assayed by development of the Porter–Silber chromogen[8]. The product was identified as 17α,21-dihydroxyprogesterone by Bush paper chromatography,[9] counter-current distribution and infrared spectroscopy.[10] The conversion of progesterone, 11β-hydroxyprogesterone and 11β,17α-dihydroxy-

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progesterone to their respective C-21 hydroxylated derivatives was also demonstrated and the products characterized by paper chromatography with several modified Bush solvent systems. The conversion of 17α-hydroxyprogesterone to Substance S is the model 21-hydroxylation reaction described in this report, but the characteristics of the system were the same for all progesterone derivatives tested.

Microsomes and 105,000g supernatant fractions of beef adrenals were found to be inactive separately but 21-hydroxylation occurred after recombination:

TABLE I

Conditions: Microsomes (20 mg. protein) and/or supernatant (94 mg. protein) were incubated in phosphate buffer pH 6.8 for one hour at 37° in air with 10 μM. DPN, 10 μM. ATP, 15 μM. niacinamide, 3 μM. 17-hydroxyprogesterone in a total of 5 cc. Incubation mixtures were extracted as described, Porter–Silber chromogen developed and measured at 410 mμ.

Fraction	Yield, μM.
1 Washed microsomes	0.00
2 105,000g supernatant	0.00
3 Recombination of 1 and 2	0.64

The 105,000g supernatant fraction from rat liver was found to be as effective as adrenal supernatant fraction when combined with the adrenal microsomes, but rat liver microsomes were inactive in recombination experiments. Treatment of the supernatant fraction with an anion exchange resin (Dowex 1-acetate form) rendered the recombined system inactive unless DPN and ATP, or TPN were added. Niacinamide also increased activity. Glucose-6-phosphate, Zwischenferment and TPN could substitute for the supernatant. Finally, TPNH in substrate amounts was equally effective and appears to be the specific coenzyme (Table II). DPNH appeared to be about one-fifth as active. Oxygen was required but cyanide and azide had no effect on the hydroxylation. Moreover, hydroxylation proceeded in the presence of catalase.

TABLE II

Conditions: additions were incubated with microsomes (34 mg. protein in case 1; 17 mg. protein in others) in Tris buffer at pH 7.2 for one hour at 37° in air with 1.0 μM./cc. 17-hydroxyprogesterone; total volume 3 cc. Incubation mixtures were extracted as described and Porter–Silber chromogen developed and measured at 410 mμ. The quantitative data are supported by chromatographic analysis.

Additions	1	2	3	4	5	6
105,000g supernatant	+					
TPN (0.3 μM./cc)	+	+		+		
Zwischenferment and glucose-6-phosphate (8 μM./cc.)		+	+			
DPNH (3 μM./cc)					+	
TPNH (3 μM./cc)						+
17,21 - Dihydroxyprogesterone formed, μM.	0.91	1.05	0.00	0.00	0.26	1.41

It is interesting to note the similarity of this adrenal system with that described by Brodie, *et al.*,[4] for the oxidation of drugs with liver microsomes and TPNH and with certain aspects of cholesterol synthesis.[3] The same fundamental mechanisms may be operative in all these cases.

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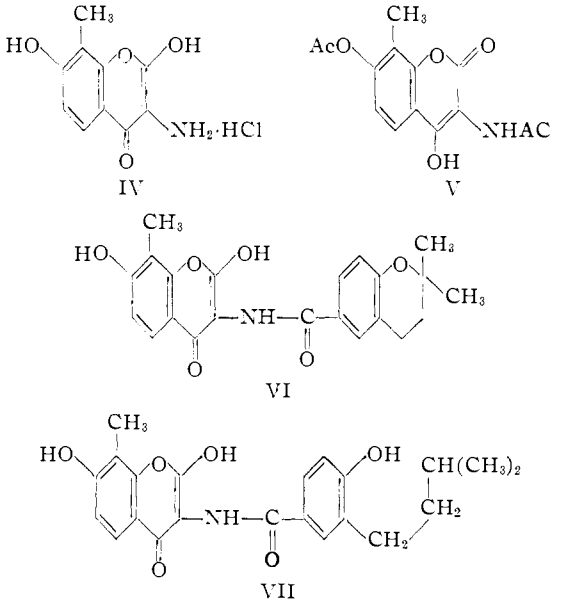
NOVOBIOCIN. IV. SYNTHESIS OF DIHYDRONOVOBIOCIC ACID AND CYCLONOVOBIOCIC ACID

Sir:

The structure of novobiocin has been elucidated by degradative studies.[1,2,3,4] We have now synthesized dihydronovobiocic acid (VII)[1] and cyclonovobiocic acid[1,2] (VI); these syntheses confirm the structure assigned to the aglycon moiety of novobiocin.

Condensation of 2-methylresorcinol with ethyl cyanoacetate in the presence of zinc chloride and hydrogen chloride gave 7-hydroxy-4-imino-8-methyl-2-oxochroman (I), m.p. > 350°.



Hydrolysis of I in 50% sulfuric acid afforded 2,7-dihydroxy-8-methylchromone (II), m.p. 270° (Calcd. for $C_{10}H_8O_4$: C, 62.50; H, 4.20. Found: C, 62.70; H, 4.45). Treatment of II with nitrous acid yielded 2,4-dioxo-7-hydroxy-8-methyl-3-oximinochroman (III), which was hydrogenated to give the amine hydrochloride IV. This compound has also been obtained by degradation.[1,2] Acetylation

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