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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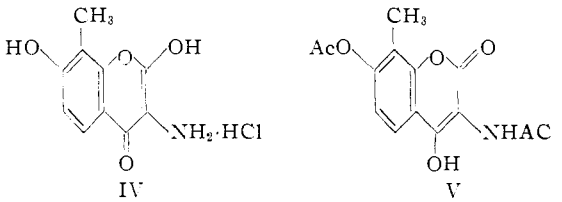
NOVOBIOCIN. IV. SYNTHESIS OF DIHYDRONOVOBIOCIC ACID AND CYCLONOVOBIOCIC ACID

Sir:

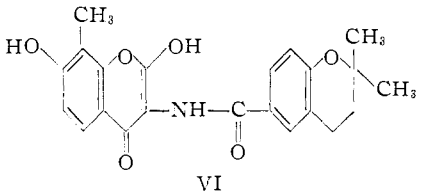
The structure of novobiocin has been elucidated by degradative studies.[1,2,3,4] We have now synthesized dihydronovobiocic acid (VII)[1] and cyclonovobiocic acid[1,2] (VI); these syntheses confirm the structure assigned to the aglycon moiety of novobiocin.

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°.

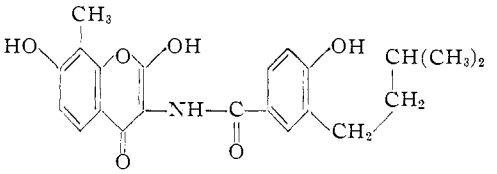


IV

V



VI



VII

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

(1) C. H. Shunk, C. H. Stammer, E. A. Kaczka, E. Walton, C. F. Spencer, A. N. Wilson, J. W. Richter, F. W. Holly and K. Folkers, THIS JOURNAL, **78**, 1770 (1956).

(2) J. W. Hinman, H. Hoeksema, E. L. Caron and W. G. Jackson, *ibid.*, **78**, 1072 (1956).

(3) E. A. Kaczka, C. H. Shunk, J. W. Richter, F. J. Wolf, M. Gasser and K. Folkers, *ibid.*, in press.

(4) H. Hoeksema, J. L. Johnson and J. W. Hinman, *ibid.*, **77**, 6710 (1955).

of IV with acetic anhydride in pyridine yielded V, m.p. 263–265° (Calcd. for $C_{14}H_{13}NO_6$: C, 57.73; H, 4.50; N, 4.81. Found: C, 57.45; H, 4.12; N, 5.10). The infrared absorption spectrum and melting point of the synthetic product and of the compound obtained by degradation of novobiocin were identical. 2,2-Dimethylchroman-6-carboxylic acid[3,4] was converted with thionyl chloride into 2,2-dimethylchroman-6-carbonyl chloride, m.p. 95–97°. Treatment of the amine hydrochloride IV with this acid chloride in pyridine gave cyclonovobiocic acid (VI), m.p. 280–284°. A mixture of this product and VI obtained by degradation of novobiocin melted at 280–286°, and the infrared absorption spectra of the two were identical.

4-Acetoxy-3-(3-methylbutyl)-benzoic acid,[4] m.p. 147–149°, was converted with thionyl chloride into 4-acetoxy-3-(3-methylbutyl)-benzoyl chloride. Upon treatment of IV with this acid chloride in pyridine, followed by hydrolysis, dihydronovobiocic acid (VII) was obtained, m.p. 237–239°. A mixture of this product and VII obtained by degradation of novobiocin melted at 237–239°. The identity was confirmed by the infrared absorption spectra.

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POLYMERIZATION INITIATED BY ELECTRON TRANSFER TO MONOMER. A NEW METHOD OF FORMATION OF BLOCK POLYMERS[1]

Sir:

Aromatic hydrocarbons react with metallic sodium in suitable solvents yielding colored and soluble complexes of composition 1 Na to 1 hydrocarbon[2] which initiate polymerization of conjugated olefinic hydrocarbons.[3] Their structure has been elucidated by Weissman and Lipkin,[4] who showed them to be composed of negative aromatic hydrocarbon ions and Na^+ ions. They have shown also that the negative hydrocarbon ions act as electron transfer agents, *e.g.*

$$\text{Naphthalene}^- + \text{Phenanthrene} \rightleftarrows \text{Phenanthrene}^- + \text{Naphthalene} \quad (1)$$

We postulate the same type of electron-transfer process to be responsible for the initiation of polymerization by sodium-naphthalene complex, *e.g.*

$$\text{Naphthalene}^- + \text{Styrene} \longrightarrow \text{Styrene}^- + \text{Naphthalene} \quad (2)$$

The negative monomer ions formed by reaction (2) may be represented formally by I or Ia.[5]

$$:CHX—CH_2\cdot \quad (I) \qquad \cdot CHX—CH_2: \quad (Ia)$$

With excess of monomer both ends of I or Ia propagate polymerization, each by a different mechanism, one end growing as a radical, the other as a carbanion. After addition of the first monomeric unit to *either* end, structures I or Ia become perfectly legitimate, *i.e.*, a true separation of electrons takes place and species like II are formed.

$$:CHX—CH_2—CHX—CH_2\cdot \text{ or } :CHX—CH_2—CH_2—CHX\cdot \quad (II)$$

The radical ends do not long exist. At low temperature they dimerise, and consequently species III are formed

$$:CHX—CH_2—CH_2—CHX: \quad (III)$$

Inspection of species III suggests that they do not terminate, and thus propagation should continue until all the monomer is consumed, and eventually a "living" polymer is produced.

The correctness of all these assumptions has been tested. Polymerization, if carried out correctly, always goes to completion. The green color of naphthalene$^-$ ion instantaneously changes into deep red on addition of styrene, the latter color due to styrene$^-$ ends. After completion of polymerization the red color persists, indicating no reverse of reaction (2). If an additional amount of styrene is added after completion of the polymerization of the first portion, the polymerization starts again, and the reaction goes again to completion.

The viscosity of the polymer solution can be measured without withdrawing the solution out of the reaction vessel. Addition of further amounts of styrene and of solvent, quantities of which are adjusted in such a way that the ratio styrene: solvent remains unaltered, leads to an increase in the viscosity of the solution. For example, styrene (9.2 g.) was added to 60 cc. of tetrahydrofuran containing 3.3×10^{-4} mole of sodium naphthalene. Polymerization was carried out at $-80°$ and after completion the viscosity of the solution was determined at room temperature (1.2–1.5 sec.). The solution was recooled to $-80°$, and an additional 7.7 g. of styrene in 50 cc. of tetrahydrofuran was added. After completion of the reaction the viscosity was found to increase to 18–20 sec. at room temperature. The final yield was 16.6 g. of polystyrene, *i.e.*, about 100% conversion. This proves conclusively the existence of living ends in these polymers, and suggests an interesting and novel method for preparation of block polymers. After completion of the first polymerization process, a second monomer is added to the still living polymers formed from the first monomer. Thus, block polymers of the type A.A···A.B.B···B.A.A···A are produced. For example, styrene (5.7 g.) was polymerized by 0.3 millimole of catalyst in the usual way and then 3.6 g. of isoprene was added. After precipitation 8.8 g. of polymer was obtained. The polymer solution in toluene could not be precipitated by isoöctane, proving the absence of pure polystyrene. On the other hand, the polymer is not extractable by isoöctane, proving the absence of pure polyisoprene. Thus, the nature of the

(1) This research was partially supported by a grant from the Office of Naval Research Contract Nonr-134091.

(2) N. D. Scott, J. F. Walker and V. L. Hansley, THIS JOURNAL, **58**, 2442 (1936).

(3) N. D. Scott, U. S. Patent 2,181,771 (1939).

(4) D. E. Paul, D. Lipkin and S. I. Weissman, THIS JOURNAL, **78**, 116 (1956).

(5) This is only a formal notation. Styrene$^-$ should be visualized as a species possessing an additional electron in the lowest π orbital which is unoccupied in the ordinary molecule of styrene.