curred since replacement of ATP with ADP yielded negligible uptake of P^{32}. On the other hand, when the enzyme preparation was incubated under strictly anaerobic conditions with β-hydroxybutyrate, the exchange was greatly inhibited. Difference spectra of the respiratory carriers[7] revealed that under the anaerobic conditions described the respiratory carriers were essentially completely reduced. Maintenance of the reduced state by addition of cyanide to inhibit cytochrome oxidase similarly led to almost complete inhibition of the exchange reaction; control experiments established that cyanide was not acting as an uncoupling agent. ATP-ase activity[8] did not change as a function of oxidation–reduction state; less than 5% of the ATP added underwent hydrolysis.

TABLE I

Expt.	Carrier state	Additions	P_i^{32} incorporated mμmoles
1	Oxidized	...	43.2
	Oxidized	10^{-4}M DNP	<0.01
	Oxidized	ADP	<0.06
	Reduced	...	9.40
	Reduced	ADP	4.02
2	Oxidized	...	32.6
	Reduced	...	8.1
3	Oxidized	...	1.27
	Reduced	...	0.10
4	Oxidized	...	20.2
	Reduced	...	1.3

All tubes contained 0.006 *M* ATP (replaced by ADP as indicated below), 0.0001 *M* P_i labeled with P^{32}, and digitonin enzyme complex in total volume of 2.0 ml.; *p*H was 6.7. In Exp. 1–4 "oxidized" systems were equilibrated with air; in Exp. 1–3 "reduced" systems contained 0.01 *M* β-hydroxybutyrate in addition to above components and the reactions were carried out anaerobically. In Expt. 4 anaerobiosis was replaced by 0.001 *M* NaCN. Reactions were started by addition of ATP and P_i^{32} and run 10–20 minutes at 25°. Incorporation data were corrected for specific activity changes. Total enzyme N added was 38.0γ in Expt. 1, 36.6γ in Expt. 2, 10.7γ in Expt. 3, and 50.0γ in Expt. 4.

These findings are consistent with a hypothesis proposed earlier,[2,3] in which reduced carrier AH_2 first reacts with a factor C to form a "low-energy" complex AH_2—C. This complex is then postulated to undergo oxidation by the next carrier B to form reduced carrier BH_2 and the "high energy" carrier complex $A_{ox} \sim C$, which then is suggested to undergo phosphorolysis followed by transfer of the ~ P so formed to ADP. The experimental findings on the ATP-P_i^{32} exchange reaction reported here are consistent with this formulation but are not consistent with the hypothesis that a complex of the reduced form[5] of the carrier is the ultimate donor of "high-energy" phosphate.

Boyer, *et al.*, have reported that the ATP-P_i^{32} exchange in intact mitochondria is not significantly altered by the presence of cyanide[9]; under the same experimental conditions we can confirm this observation. However with lower concentrations of mitochondria and with special precautions to insure complete reduction of the carriers in the presence of β-hydroxybutyrate and cyanide, prior to addition of ATP and P_i^{32}, the inhibition of the exchange reaction is nearly complete.

(9) P. D. Boyer, W. W. Luchsinger and A. B. Falcone, *J. Biol. Chem.*, **223**, 405 (1956).

This investigation was supported by the U.S. Public Health Service, the National Science Foundation, and the Nutrition Foundation, Inc. A U.S. Public Health Service Postdoctoral Fellowship (C.L.W.) is gratefully acknowledged.

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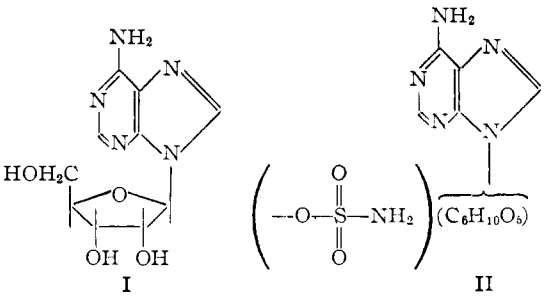
RECEIVED JANUARY 2, 1957

THE STRUCTURE OF NUCLEOCIDIN. I.

Sir:

The isolation,[1a] physical properties,[1a] and phenomenal anti-trypanosomal activity[1b] of nucleocidin have been reported recently. We now describe structural studies which show that nucleocidin is a glycoside of adenine in which sulfamic acid is bound to the carbohydrate moiety as an ester.

When hydrolyzed with ethanolic hydrochloric acid[2] the antibiotic yielded adenine, which was characterized as its sulfate and picrate. The ultraviolet spectrum of nucleocidin is nearly superimposable on the spectrum of adenosine (I) in either acid or alkali; since the ultraviolet spectra of adenine derivatives vary according to the location



I II

of substituents on the purine skeleton,[3,4] nucleocidin must be presumed to be a 9-adenyl compound.

The conditions required for acid hydrolysis, the high proportion of oxygen in the molecule, and analogy to other "nucleoside-type" antibiotics[2,5,6] all suggested that nucleocidin was a glycoside of adenine. As expected, the antibiotic yielded a reducing sugar after acid hydrolysis: the hydrolyzate gave a positive Fehling test, a precipitate with phenylhydrazine, and a spot test color with aniline phthalate.

Hydrolysis (100°, 30 min.) of the antibiotic with 2*N* hydrochloric acid containing barium chloride produced barium sulfate. The sulfur atom in nucleocidin is, therefore, hexavalent, and not linked directly to carbon, since sulfones and alkane sulfonic acids are stable under these conditions.

After hydrolysis of nucleocidin using Dowex-

(1) (a) S. O. Thomas, V. L. Singleton, J. A. Lowery, R. W. Sharpe, L. M. Pruess, J. N. Porter, J. H. Mowat and N. Bohonos, *Antibiotics Annual*, 1956–1957, in press. (b) R. I. Hewitt, A. R. Gumble, L. H. Taylor, W. S. Wallace, *ibid.*, in press. These two papers are the texts of reports read before the Fourth Annual Antibiotic Symposium, Washington, D. C., October 17–19, 1956.

(2) *Cf.* C. W. Waller, P. W. Fryth, B. L. Hutchings and J. H. Williams, THIS JOURNAL, **75**, 2025 (1953).

(3) J. M. Gulland and E. R. Holiday, *J. Chem. Soc.*, 765 (1936).

(4) J. M. Gulland, R. F. Story, *ibid.*, 259 (1938).

(5) H. R. Bentley, *et al.*, *ibid.*, 2303 (1951).

(6) N. Löfgren, B. Lüning and H. Hedström, *Acta Chem. Scand.*, **8**, 670 (1954).

50[7,8] followed by paper chromatography of the hydrolyzate in an ethanol-ammonia-water system,[9] sulfamic acid could be identified by its R_f value (0.34–0.37) and by its specific reaction with a nitrous acid–benzidine spray devised especially for this purpose.

The antibiotic has pK_a 9.3 as an acid, too high for a free sulfonic acid group, but of the same order of magnitude as the pK_a of a sulfonamide. Treatment of nucleocidin with barium nitrite in dilute acid at room temperature produced barium sulfate where treatment with barium chloride under the same conditions leaves the antibiotic unaffected. From the foregoing evidence we conclude that nucleocidin is not an amine sulfonate but an ester of sulfamic acid.

Nucleocidin, $C_{11}H_{16}N_6SO_8$,[1a] should, therefore, be formulated as II. Except for a few simple synthetic examples[10] no esters of N-unsubstituted sulfamic acid have been described previously. The unusual structure of the carbohydrate moiety of nucleocidin and the total structure of the antibiotic will be reported in a separate communication.

(7) J. X. Khym, *et al.*, THIS JOURNAL, **75**, 1262 (1953).

(8) J. X. Khym and W. E. Cohn, *ibid.*, **76**, 1818 (1954).

(9) A. G. Long, J. Quayle and R. J. Stedman, *J. Chem. Soc.*, 2197 (1951).

(10) Even the claims for simple alkyl sulfamates seem open to question. For references in this confused field, see: (a) R. Behrend, *Ann.*, **222**, 116 (1884); (b) W. W. Binkley and E. F. Degering, *Proc. Indiana Acad. Sci.*, **47**, 122 (1938); (c) **53**, 122 (1943); (d) R. Kuhn and W. Brydowna, *Ber.*, **70**, 1333 (1937); (e) W. Traube, H. Zander and H. Gaffron, *ibid.*, **57**, 1045 (1924).

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RECEIVED JANUARY 19, 1957

CHOLESTEROL—A PRECURSOR OF ESTRONE *IN VIVO*

Sir:

Following the report by Heard and O'Donnell[1] of the inability of the pregnant mare to synthesize estrone from C^{14}-labeled cholesterol, it was assumed that this sterol was not a precursor of the estrogen hormones.[2] Recently, however, evidence to the contrary has accumulated. The conversion of C^{14}-labeled testosterone[3–5] and 19-hydroxy-Δ^4-androstene-3,17-dione[6] to the estrogens have strongly implicated cholesterol as a precursor. We wish to record an experiment that demonstrates this conversion. A pregnant woman[7] was given 87.4 μc. of cholesterol-4-C^{14} (2.35 μc./mg.) over a six-day period during which time daily urine samples were collected and assayed for radioactivity (0.6% found). The urinary steroid conjugates were hydrolyzed with β-glucuronidase and extracted with ether at *p*H 5. The crude phenolic fraction, weighing 338 mg. and with 0.012% of the administered radioactivity, was obtained by using the procedure of Engel.[8] Carrier estrone, 3.875 mg. was added to the toluene before the extraction of the estrogens.[8] The ketonic residue from a Girard separation was chromatographed for 3 hours on 15 sheets of Whatman no. 1 paper in the methanol–water–benzene–petroleum ether system of Migeon, *et al.*[9] The area with an R_f corresponding to estrone was cut out and eluted with methanol. Using the Kober assay, 4.5 mg. were found. The material did not separate from authentic estrone in mixed paper chromatography in two solvent systems.[10] Estrone, 110.9 mg., was added as carrier and the acetate was prepared in the usual manner. The acetate was purified over charcoal and crystallized twice from aqueous methanol. The crystals were dissolved in 15 ml. of toluene containing 30 mg. of 2,5-diphenyloxazole (DPO) and the solution was counted in a Packard Tri-Carb Liquid Scintillation Counter.[11] The toluene was removed *in vacuo* and the acetate was freed from DPO by washing twice with 0.5 ml. of petroleum ether. After two crystallizations from aqueous methanol, the acetate was recounted. The toluene was then removed, the acetate hydrolyzed with 5% methanolic potassium hydroxide, and the estrone converted to the benzoate derivative.[3] The benzoate was crystallized from 95% ethanol twice (15 ml. each time) and was counted as described above for the acetate. After the toluene was removed and the crystallization steps again carried out, the counting was repeated. The counting data are shown in Table I. It is unlikely that the estrone was synthesized from a degradation product of cholesterol-4-C^{14} since this sterol does not appear to be degraded *in vivo* to any appreciable extent.[12]

TABLE I

SPECIFIC ACTIVITY OF ESTRONE ISOLATED BY CARRIER TECHNIQUE

Derivative	No. times crystallized	M.p.°[a]	Mg. counted	DPM[b]	DPM/mg. free estrone
Acetate	2	125.5–127.0	117.4	1615	15.9
Acetate	4	120.5–121.5	96.1	1335	16.0
Benzoate	2	217.0–219.5	84.7	899	14.7
Benzoate	4	216.5–218.5	77.0	806	14.5
Benzoate	6	216.0–218.0	68.9	761	15.3

[a] Taken on a Fisher-Johns apparatus, uncorrected. [b] Corrected for quenching effects (23–30%) on the counting. The counting efficiency was 61.5% and the background was 35 c.p.m.

When the placenta was removed the venous plasma free cholesterol specific activity was 1871 DPM/mg., while that of the placenta cholesterol (free + ester) was 816 DPM/mg. Although the data do not permit a determination of the fraction

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(2) S. Roberts and C. M. Szego, *Ann. Rev. Biochem.*, **24**, 557 (1955).

(3) R. D. H. Heard, P. H. Jellnick and V. J. O'Donnell, *Endocrinology*, **57**, 200 (1955).

(4) H. W. Wotiz, J. W. Davis, H. M. Lemon and M. Gut, *J. Biol. Chem.*, **222**, 487 (1956).

(5) B. Baggett, L. L. Engel, K. Savard, and R. I. Dorfman, *ibid.*, **222**, 931 (1956); *Fed. Proc.*, **14**, 175 (1955).

(6) A. S. Meyer, *Biochem. Biophys. Acta*, **17**, 441 (1955).

(7) Six days before a scheduled therapeutic abortion.

(8) L. L. Engel, *Recent Progress Hormone Research*, **5**, 343 (1950).

(9) C. J. Migeon, W. R. Slaunwhite, Jr., R. Aldous, R. Fox, R. Hardy, D. Johnson and W. Perkins, *J. Clin. Endocrinol. & Metab.*, **15**, 775 (1955).

(10) Toluene–propylene glycol[8] and that described by Migeon *et al.*[9]

(11) Commercially available from Packard Instrument Corp., LaGrange, Illinois.

(12) H. Werbin, D. M. Bergenstal, R. G. Gould and G. V. LeRoy, *J. Clin. Endocrinol. & Metab.*, in press.