nine evenly spaced lines with approximately binomial intensity distribution. The interval between lines is 2.7 oersteds. $[2.2]^-$ has a poorly resolved spectrum of at least nine components. $[4.4]^-$ and $[6.6]^-$ have indistinguishable spectra of five evenly spaced lines with binomial intensity distribution. The lines are completely resolved and are separated by 5.5 oersteds. $[3.4]^-$ has a spectrum resembling those of $[4.4]^-$ and $[6.6]^-$ but with wider lines and broad tails extending beyond the 22 oersted spread of the former. The spectra may be interpreted as follows: In $[4.4]^-$ and $[6.6]^-$ the electron exchange proceeds less frequently than 3×10^6 sec.$^{-1}$ (the line breadth in frequency), while in $[1.8]^-$ and $[2.2]^-$ it proceeds more rapidly than 1.5×10^7 sec.$^{-1}$ In $[3.4]$ the exchange rate is somewhat higher than 3×10^6 sec.$^{-1}$ but less than 1.5×10^7 sec.$^{-1}$.

Further work on other paracyclophanes and on the temperature dependence of the rates is in progress.

I am deeply indebted to Professor Cram for all the paracyclophanes which have been used in these experiments.

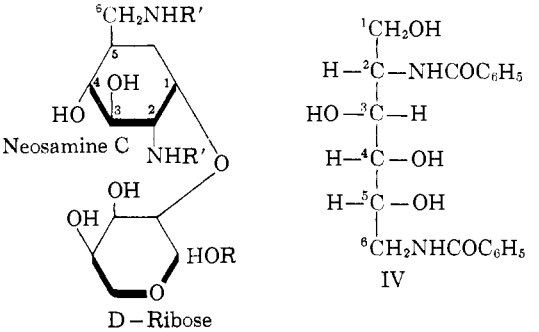
DEPARTMENT OF CHEMISTRY
WASHINGTON UNIVERSITY S. I. WEISSMAN
SAINT LOUIS, MISSOURI

RECEIVED OCTOBER 4, 1958

CHEMISTRY OF THE NEOMYCINS. III. THE STRUCTURE OF NEOBIOSAMINE C

Sir:

It was reported recently[1,2] that neobiosamine C, approximately one-half of neomycin C, is a disaccharide composed of a diaminohexose (for which we now propose the name neosamine C, *cf.* accompanying Communication)[3] and D-ribose. We present here evidence showing neobiosamine C to have the structure I.



I, R = R' = H (Neobiosamine C)
II, R = CH_3, R' = COC_6H_5
III, R = CH_3, R' = H

Cleavage of the glycosidic linkage in methyl N,N'-dibenzoylneobiosaminide C[1] (II) by mild acidic hydrolysis,[2] followed by sodium borohydride reduction, gave N,N'-dibenzoylneosaminol C (IV), m.p. 186–188.5°. [Found: C, 61.80; H, 6.23; N, 7.28], which reduced two moles of sodium periodate with formation of no formaldehyde. The periodate product was oxidized with bromine water, then hydrolyzed vigorously, to give glycine [papergram—violet ninhydrin spot, R_f 0.445 (PyW),[4] 0.375 (PhenW)[4]; authentic sample—violet, R_f 0.446 (PyW), 0.363 (PhenW)] and serine [purple spot, R_f 0.531 (PyW), 0.305 (PhenW); authentic sample—purple, R_f 0.528 (PyW), 0.286 (PhenW)]. Methyl N,N'-dibenzoylneobiosaminide C(II) consumes two moles of periodate, thus must have one —CHOHCHOH— grouping in each monosaccharide (ribose and neosamine) moiety. This periodate oxidation product was treated with bromine water, then hydrolyzed vigorously to give isoserine [papergram—yellow ninhydrin spot, R_f 0.43 (PyW), 0.27 (PhenW); authentic sample—yellow, R_f 0.42 (PyW), 0.26 (PhenW)], as well as glycine [violet spot, R_f 0.39 (PyW), 0.34 (PhenW); authentic sample—violet, R_f 0.39 (PyW) 0.33 (PhenW)]. The formation of isoserine establishes an aldohexose (rather than a ketohexose) structure for neosamine C, while demonstration of a vicinal glycol grouping in this moiety of II shows neosamine C to contain a pyranose (rather than furanose) ring in I. The structure of neosamine C in neomycin C is, therefore, that shown in I.

Methyl neobiosaminide C[1] (III) was hydrolyzed under mild conditions to I, which was reduced with sodium borohydride, then N-benzoylated to give N,N'-dibenzoylneobiosaminol C, m.p. 217–227° dec. [Found: C, 56.12; H, 6.31; N, 5.10]. Vigorous hydrolysis of this derivative gave ribitol [papergram—colorless with aniline acid phthalate,[5] R_f 0.616 (BAW 221)[4]; authentic sample—colorless, R_f 0.612 (BAW 221)], thus confirming the earlier assignment[1] of neobiosamine C as a neosaminidoribose, rather than a ribosidoneosamine, which would have given ribose [authentic sample—red spot, R_f 0.584 (BAW 221)]. N,N'-Dibenzoylneobiosaminol C consumed three moles of sodium periodate, with formation of one mole of acid. A glycosidic bond to the ribose C-5 position would require a four-mole periodate uptake, with formation of two moles of acid. The ribose C-3 position is eliminated *per se* by the uptake of one mole of periodate in the ribose moiety of methyl N,N'-dibenzoylneobiosaminide C (*cf.* above), (impossible with a C-3 link since two adjacent hydroxyl groups are prohibited for either pyranose or furanose forms). The ribose C-4 position is also extremely unlikely, since oxidation of neobiosamine with bromine water gave a product whose infrared spectrum contains a carbonyl band at 1765 cm.$^{-1}$, indicative of a γ-lactone,[6] hence of a free C-4 hydroxyl. The sole remaining ribose position is C-2 and the structure of neobiosamine C is I.

This investigation was supported in part by a research grant, No. E-1278, from the National Institute of Allergy and Infectious Diseases, Public Health Service. We also wish to express our thanks to the Upjohn Company for the generous

(1) K. L. Rinehart, Jr., P. W. K. Woo, A. D. Argoudelis and A. M. Giesbrecht, THIS JOURNAL, **79**, 4567 (1957).

(2) K. L. Rinehart, Jr., P. W. K. Woo and A. D. Argoudelis, *ibid.*, **79**, 4568 (1957).

(3) K. L. Rinehart, Jr., P. W. K. Woo and A. D. Argoudelis, *ibid.*, **80**, 6461 (1958).

(4) PyW is 97.5 parts pyridine +52.5 parts water. PhenW is phenol saturated with an aqueous solution containing 3.7% sodium dihydrogen phosphate and 6.3% sodium citrate. BAW 221 is *tert*-butyl alcohol:acetic acid:water:2:2:1.

(5) S. M. Partridge, *Nature*, **164**, 443 (1949).

(6) S. A. Barker, E. J. Bourne, R. M. Pinkard and D. H. Whiffen, *Chem. and Ind. (London)*, 658 (1958).

gift of neomycin samples, and to Mr. A. D. Argoudelis for counsel and assistance.

(7) Robert F. Carr Fellow, 1957–58.

DEPARTMENT OF CHEMISTRY
AND CHEMICAL ENGINEERING
UNIVERSITY OF ILLINOIS KENNETH L. RINEHART, JR.
URBANA, ILLINOIS PETER W. K. WOO[7]

RECEIVED SEPTEMBER 9, 1958

A NEW CLASS OF POTENT CORTICAL HORMONES.[1] 6α-CHLOROCORTICOIDS

Sir:

We have found that 6β-chloro substitution among corticoids decreases thymolytic and anti-inflammatory activity and increases sodium retention, 6α-chloro substitution in general not only enhances thymolytic and anti-inflammatory activity but also modifies biological activity toward sodium excretion. Thus 6α-chloroprednisone acetate (A^2 = 4) and 6α-chloroprednisolone (A^2 = 13) exhibit a marked sodium excretion[3] while 6α-chloro-9α-fluoroprednisolone acetate (A = 40) is only a moderate sodium retainer in contrast to the enormous sodium retention of the C-6 unsubstituted compound.[4]

6α - Chloro - 9α - fluoro - 16α - hydroxyhydrocortisone 21-acetate 16,17-acetonide (XI) and the corresponding 1-dehydro compound (XIV), with respective anti-inflammatory activities[2] of 50 and 200 × hydrocortisone exhibit sodium excretion.

The 3-ethyl enol ether[5] of Reichstein's "S" acetate on reaction with N-chloroacetamide in aqueous buffered acetone solution yielded 6β-chloro-"S" acetate (m.p. 193–194°, $[\alpha]_D$ +41°, λ_{max} 240 mμ, log ε 4.15. Found for $C_{23}H_{31}ClO_5$: C, 65.38; H, 7.41; Cl, 8.28) which was inverted to XV by treatment with hydrogen chloride in acetic acid. Selenium dioxide oxidation[6a,b,c,d] followed by saponification and (1) adrenal incubation[7] or (2) fermentation with *Cunninghamella*[8] *bainieri* ATCC 9244 gave 6α-chloroprednisolone (XII). Adrenal or microbiological oxidation of XV followed by acetylation or hydrogen chloride–acetic acid treatment of 5α,6α-oxido - 3,20 - bisethylenedioxyallopregnane - 11β,-17α,21-triol[9] acetate gave VIII which was converted to XII acetate (m.p. 204–205°, $[\alpha]_D$ +68°, λ_{max} 242 mμ, log ε 4.17. Found for $C_{23}H_{29}ClO_6$: C, 63.01; H, 6.68; Cl, 7.99) by selenium dioxide oxidation.

TABLE I

	Compound[b]	Additional substituent	M.P. (dec.) °C.	$[\alpha]_D$ $CHCl_3$	λ_{max} (mμ) EtOH	log ε	Thymolytic[a] activity	Anti-inflamm.[2,a] activity
I	Cortisone Ac.	6β-Chloro-	178–179°	+129°	237	4.16	0.2	0.2
II	Cortisone Ac.	6α-Chloro-	197°	+176°	233	4.16	1.3	1
III	Cortisone Ac.	6β-Chloro-9α-fluoro	221°	+ 80°	234	4.18	1.5	1
IV	Cortisone Ac.	6α-Chloro-9α-fluoro	214°	+132°	230	4.16	15	8
V	Prednisone Ac.	6β-Chloro-	221–222°	+132°	241	4.17	0.3	0.3
VI	Prednisone Ac.	6α-Chloro-	217–218°	+144°	237	4.19	4	4
VII	Prednisone Ac.	6α-Chloro-9α-fluoro	227–229°	+122°	235	4.18	18	14
VIII	Hydrocortisone Ac.	6α-Chloro	174–176°[c]	+ 97	238	4.08	5	11
IX	Hydrocortisone Ac.	6β-Chloro-9α-fluoro	194–195°	+ 44°	238	4.17	0.5	0.5
X	Hydrocortisone Ac.	6α-Chloro-9α-fluoro	134–143°[d]	+ 88°	234	4.11	5	11
XI	Hydrocortisone Ac.	6α-chloro-9α-fluoro-16α-hydroxy-16,17-acetonide	156–157°	+ 90°	234	4.17	100	50
XII	Prednisolone	6α-Chloro	195–196°	+ 61° (Diox.)	242	4.19	14	13
XIII	Prednisolone Ac.	6α-Chloro-9α-fluoro	150°[d,e]	+ 71°	238	4.19	27	40
XIV	Prednisolone Ac.	6α-Chloro-9α-fluoro-16α-hydroxy-16,17-acetonide	294–296°	+ 53°	238	4.17	400	200
XV	"S" Acetate	6α-Chloro	189–190°	+ 87°	237	4.18		
XVI	Δ^1-Dehydro "S" Ac.	6α-Chloro	231–232°	+ 41°	243	4.21		

[a] Thymolytic activity in adrenalectomized rat, oral route, hydrocortisone acetate = 1. Anti-inflammatory assay in immature adrenalectomized rat, cotton pellet implant. Assays by R. I. D. [b] Correct elemental analyses were obtained for all compounds. [c] Double m.p. 105° and 174–176°. [d] Amorphous. [e] Contracts at 110°.

Representative of the synthesis of I, II, III, IV, V, VI, VII, IX, X and XIII is the preparation of XI and XIV. 9α-Fluoro-16α-hydroxyhydrocortisone 16,17-acetonide 21-acetate[10] was converted to the 3-ethyl enol ether (m.p. 214–215°, $[\alpha]_D$ −5°, λ_{max} 241 mμ, log ε 4.31. Found for $C_{28}H_{39}FO_7$: C, 66.23; H, 7.73) which reacted with N-chlorosuccinimide to give the 6β-chloro-Δ^4-3-ketone (m.p. 185–186°, $[\alpha]_D$ +63°, λ_{max} 238 mμ, log ε 4.12. Found for $C_{26}H_{34}ClFO_7$: C, 60.58; H, 6.67; Cl, 6.62). Hydrogen chloride inversion

(1) Paper CVII, J. Iriarte, C. Djerassi and H. J. Ringold, THIS JOURNAL, **81**, in press (1959).

(2) A = Anti-inflammatory activity, oral route, hydrocortisone acetate = 1.

(3) Salt assay in adrenalectomized rat without sodium chloride load.

(4) J. Fried, K. Florey, E. F. Sabo, J. E. Herz, A. R. Restivo, A. Borman, and F. M. Singer, THIS JOURNAL, **77**, 4181 (1955).

(5) P. L. Julian, E. W. Meyer, W. J. Karpel and W. Cole, *ibid.*, **73**, 1982 (1951).

(6) (a) H. J. Ringold, G. Rosenkranz and F. Sondheimer, *J. Org. Chem.*, **21**, 239 (1956); (b) Ch. Meystre, H. Frey, W. Voser and A. Wettstein, *Helv. Chim. Acta*, **39**, 734 (1956); (c) S. A. Szpilfogel, T. A. P. Posthumus, M. S. De Winter and D. A. Van Dorp, *Rec. trav. chim.*, **75**, 475 (1956); (d) K. Florey and A. R. Restivo, *J. Org. Chem.*, **22**, 406 (1957).

(7) A. Zaffaroni, H. J. Ringold, G. Rosenkranz, F. Sondheimer, G. H. Thomas and C. Djerassi, THIS JOURNAL, **80**, 1958.

(8) *Cf.* D. H. Peterson, *Record of Chemical Progress*, **17**, 211 (1956), for a review of related microbiological oxidations.

(9) R. Littell and S Bernstein, THIS JOURNAL, **78**, 984 (1956).

(10) J. Fried, A. Borman, W. B. Kessler, P. Grabowich, E. F. Sabo, *ibid.*, **80**, 2338 (1958).