More recently, Dessy, Wotiz, Hollingsworth and co-workers have described experiments which have indicated that the species RMgX is essentially non-existent in the Grignard equilibria. The basis for this conclusion was the lack of exchange of Mg between $Mg^{28}Br_2$ and Et_2Mg in ether solution.[2] Evidence was presented showing that an equimolar mixture of $MgBr_2$ and Et_2Mg has the same characteristics as the Grignard reagent.[3,4,5] Thus, it was concluded that the Grignard reagent is best represented by the equilibrium

$$R_2Mg + MgX_2 \leftrightarrows R_2Mg \cdot MgX_2$$

Since Grignard reagents in diethyl ether solution are dimeric (based on RMgX), and $MgCl_2$ does not precipitate from a solution of EtMgCl on standing, the above equilibrium is said to lie far to the right.

Recently we have made two unusual observations concerning the nature of the Grignard reagent which have caused us to reconsider the equilibrium proposed by Dessy: (1) the Grignard reagent,[6] although dimeric in diethyl ether, is monomeric in tetrahydrofuran, and (2) fractional crystallization of "EtMgCl" in tetrahydrofuran produced $EtMg_2Cl_3$ and Et_2Mg in quantitative yield. These observations have led directly to two conclusions: (1) in tetrahydrofuran there *is* alkyl exchange in Grignard solutions, and (2) the predominant species in solution is RMgX.

The monomeric nature of "EtMgCl" in tetrahydrofuran implies that the species in solution are (1) EtMgCl, (2) a mixture of Et_2Mg and $MgCl_2$, or (3) an equilibrium mixture of (1) and (2). The possibility of (2) seems unlikely since a 2 *M* solution of "EtMgCl" did not precipitate $MgCl_2$ on standing although the solubility limit of $MgCl_2$ in tetrahydrofuran is only 0.5 *M*. Confirmation of the lack of association of the predominant species in solution was given by the observation that both Et_2Mg and $MgCl_2$ were found to be monomeric in tetrahydrofuran. Consequently, the implication is strong that in tetrahydrofuran the Grignard reagent must be predominantly in the form originally ascribed to it by Schlenk,[7] namely, RMgX. Thus the equilibrium

$$RMgX \leftrightarrows R_2Mg + MgX_2$$

is supported with the equilibrium lying predominantly to the left. The equilibrium could be extended to include RMg_2X_3.

$$3\ RMgX \leftrightarrows 3/2\ R_2Mg + 3/2\ MgX_2 \leftrightarrows RMg_2X_3 + R_2Mg$$

However, it is felt that the RMg_2X_3 is formed on crystallization through a combination of RMgX and MgX_2 in the tetrahydrofuran–benzene system.

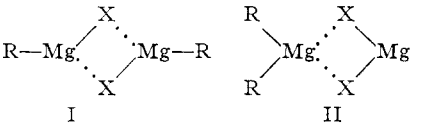
The molecular association of EtMgCl and EtMgBr was determined ebullioscopically in tetrahydrofuran at the normal boiling point of the solution (66°) and at 30°. The values listed are the average values of

	Concentration, moles/liter	760 mm. (66°)	200 mm. (30°)
EtMgBr	0.1–0.3	1.01	1.04
EtMgCl	0.1–0.3	1.11	1.01

three consecutive determinations. The molecular weight of a 2 *M* solution of EtMgCl in tetrahydrofuran was shown to be 65 (theory, 88.8). Although molecular weight determinations at such a high concentration are not too accurate, it appears that "EtMgCl" even at this concentration is essentially monomeric.

The fractional crystallization of EtMgCl from tetrahydrofuran was accomplished by adding an equal volume of benzene to the Grignard solution and slowly removing the solvent mixture under vacuum. Two fractions of white crystalline solids were isolated. Magnesium, chlorine and gas evolution analyses determined the empirical formula of both fractions to be $EtMg_2Cl_3 \cdot THF$. The solute in the mother liquor was shown by analysis to be Et_2Mg.

In contrast, we have found the molecular weight of EtMgCl in diethyl ether to indicate a dimeric structure over a wide concentration range. The two most logical dimeric structures are shown as I and II. The work



I II

of Dessy and co-workers leads to preference of structure II over structure I.

Fractional crystallization of EtMgCl and MeMgCl from diethyl ether solution did not produce the same clear-cut results as did the experiments carried out in tetrahydrofuran. However, $MeMg_2Cl_3$ was isolated in good yield from an ether solution of the Grignard prepared from methyl chloride and magnesium, thus showing that alkyl exchange also occurs in diethyl ether. With alkyl exchange demonstrated, there is no reason to prefer the unsymmetrical structure (II) proposed by Dessy over the symmetrical one (I), especially in view of the results obtained in tetrahydrofuran. In this connection we have determined the molecular aggregation of mesitylmagnesium bromide in diethyl ether to be dimeric. If Grignard compounds in diethyl ether solution exist as the unsymmetrical dimers, a severe steric problem would arise from a magnesium atom surrounded by two mesityl groups and two bromine atoms. Thus, it would appear that the equilibrium which exists in diethyl ether is similar to that occurring in tetrahydrofuran.

We are continuing to study the relationship of structure to molecular association in Grignard solutions. Work is also in progress to further define the significance and role of the RMg_2X_3 species in Grignard solution.

The support of the Advance Research Projects Agency, Department of Defense, under whose sponsorship this work was conducted, is gratefully acknowledged.

RESEARCH AND DEVELOPMENT DEPARTMENT
ETHYL CORPORATION
BATON ROUGE 1, LOUISIANA
E. C. ASHBY
W. E. BECKER

RECEIVED OCTOBER 25, 1962

(2) R. E. Dessy, G. S. Handler, J. H. Wotiz and C. A. Hollingsworth, *J. Am. Chem. Soc.*, **79**, 3476 (1957).

(3) R. E. Dessy, J. H. Wotiz and C. A. Hollingsworth, *ibid.*, **79**, 358 (1957).

(4) R. E. Dessy and R. M. Jones, *J. Org. Chem.*, **24**, 1685 (1959).

(5) R. E. Dessy, *ibid.*, **25**, 2260 (1960).

(6) All Grignard solutions prepared in either tetrahydrofuran or ether, were prepared by the reaction of the alkyl halide with magnesium. In tetrahydrofuran the solution prepared from ethyl chloride and magnesium was identical to the solution prepared from Et_2Mg and $MgCl_2$ with respect to infrared spectra, conductance and dipole moment.

(7) W. Schlenk and W. Schlenk, Jr., *Ber.*, **62**, 920 (1929).

THE CHEMISTRY OF BLUENSOMYCIN. I.[1a] THE STRUCTURE OF BLUENSIDINE

Sir:

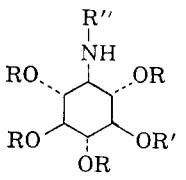
The isolation of a new basic antibiotic, bluensomycin, has been described recently.[1b] Bluensomycin purified as its hydrochloride, has the molecular formula $C_{21}H_{39}N_5O_{14} \cdot 2HCl$,[2] pK_a' 7.53, equivalent weight 659. It gave a strong Sakaguchi color test, indicative of a monosubstituted guanidino group. The hydrochloride showed infrared absorption at 3350–2950, 1710, 1655, 1610,

(1a) Bluensomycin is the generic name for antibiotic U-12,898.

(1b) M. E. Bergy, T. E. Eble, R. R. Herr, C. M. Large and B. Bannister, Second Interscience Conference on Antimicrobial Agents and Chemotherapy, Oct. 31–Nov. 2, 1962, Chicago, Illinois.

(2) Analytical values for all the compounds described in this paper were consistent with the indicated formulas.

1140–960, 850, and 760 cm.$^{-1}$, and no ultraviolet absorption. Functional group determination and n.m.r. spectrum indicated one C-CH_3 and one N-CH_3 group, the latter assignable to the titrable base. Methanolysis of bluensomycin hydrochloride in anhydrous *N* methanolic hydrogen chloride at room temperature was shown by paper chromatography to result in the cleavage of the molecule into two parts. Carbon chromatography of the reaction mixture gave, by elution with water, a strong base designated bluensidine and, by elution with 10% acetone, the remainder of the molecule as a larger fragment, which is discussed in a subsequent communication.[3] In this paper I is presented as the structure for bluensidine. Bluensidine (I) was isolated as its hydrochloride, $C_8H_{16}N_4O_6 \cdot HCl$, m.p. 190–194° (dec.), $[\alpha]^{24}D$ + 0.5 to 1.5° (*c*, 1, water). The presence of a guanidino group in I was suggested by the strong basic properties, the positive Sakaguchi test and the presence of an absorption band at 1670 cm.$^{-1}$ in the infrared spectrum. In addition to the 1670 cm.$^{-1}$ absorption, the infrared spectrum showed carbonyl absorption at 1710 cm.$^{-1}$ and bands at 3500–3300 cm.$^{-1}$, indicative of the presence of hydroxyl or amino hydrogen. Hydrolysis of I under reflux with saturated barium hydroxide for 18 hr., gave two moles of carbon dioxide



I, R = H, R′ = $-CONH_2$, R″ = $-C(=NH)-NH_2$
II, R = R′ = R″ = H
III, R = R′ = R″ = $-COCH_3$
IV, R = R′ = H, R″ = $-COCH_3$
V, R = R′ = H, R″ = $-CONH_2$
VI, R = R′ = H, R″ = $-C(=NH)-NH_2$
VII, R = $-COCH_3$, R′ = $-CONH_2$, R″ = $-C(=NHCOCH_3)-NCOCH_3$

(isolated as barium carbonate), three moles of ammonia, and an optically inactive base (II), pK_a' 7.5 (water), crystalline as its hydrochloride $C_6H_{13}NO_5 \cdot HCl$, which darkens at 265° but does not melt below 300°. Van Slyke nitrogen determination indicated one primary amino group. The base (II) consumed 6 moles of periodate per mole with no formation of formaldehyde. These data point to an amino-pentahydroxycyclohexane structure for II. The lack of infrared absorption in the 900–800 cm.$^{-1}$ region suggested[4] the all-*trans* configuration, *i.e.*, the identity of II to *scyllo*-inosamine. This was confirmed by comparison[5] of the hexaacetyl (III) and the N-acetyl (IV) derivatives of II to authentic samples[6] of hexaacetyl-*scyllo*-inosamine and N-acetyl-*scyllo*-inosamine respectively.[7,8] Mild alkaline hydrolysis of I gave two moles of ammonia, one mole of carbon dioxide and an optically inactive, neutral crystalline compound, bluensurea (V), $C_7H_{14}N_2O_6$, m.p. 244–245° (dec.). Strong alkaline hydrolysis of V afforded II together with one mole of carbon dioxide and one mole of ammonia. Acid hydrolysis of I gave one mole each of carbon dioxide and ammonia plus an optically inactive strong base (VI) isolated as its crystalline hydrochloride, $C_7H_{15}N_3O_5 \cdot HCl$, m.p. 228–230° (dec.). The infrared absorption at 1710 cm.$^{-1}$ was absent in both V and VI. Only VI gave a positive Sakaguchi test. Strong alkaline hydrolysis of VI afforded II with the formation of one mole of carbon dioxide and two moles of ammonia, and VI can thus be formulated as 1-deoxy-1-guanidino-*scyllo*-inositol. The degradation of I to VI with the simultaneous formation of one mole of carbon dioxide and one mole of ammonia, when considered with the 1710 cm.$^{-1}$ absorption, suggests the presence of a primary carbamoyl group in I. Hence, I is a guanidino-O-carbamoyl-*scyllo*-inositol. In confirmation of this structure, acetylation of I in acetic anhydride and pyridine afforded a crystalline hexaacetyl derivative, $C_{20}H_{28}N_4O_{12}$ (VII), m.p. 250–251° (dec.), $[\alpha]^{25}D$ + 5° (*c*, 0.9, chloroform), which had carbonyl absorption at 1710 cm.$^{-1}$ in addition to the ester carbonyl absorption at 1740 cm.$^{-1}$. Ammonolysis of VII in methanol afforded I. The n.m.r. spectrum[9,10] of VII showed four types of hydrogens. A broad multiplet at 780 c.p.s. of area 1 and a doublet at 551, 542 c.p.s. of total area 1 correspond to the hydrogens on N^3 and N^1 of the guanidino group. A complex multiplet at 345–275 c.p.s. of area 8 corresponds to the six ring hydrogens plus the two carbamoyl hydrogens.[11] Finally, five peaks at 128, 125, 122, 121 and 117 c.p.s. of total area 18 correspond to the 6 C-CH_3 of the acetyl groups. Aside from considerations of absolute stereochemistry, the only possible isomers of I are those having the guanidino and the carbamoyl group 1,2-, 1,3- or 1,4 to each other. From symmetry considerations, the optical activities of I and VII exclude the 1,4 arrangement. That bluensidine is 1-deoxy-1-guanidino-3-O-carbamoyl-*scyllo*-inositol was shown by periodate oxidation studies, in which I consumed 2 moles of periodate per mole, forming 1 mole of formic acid. Only the 1,3 arrangement is consistent with this, establishing I as the structure of bluensidine. The problem of determining the absolute configuration in bluensidine is under study and will be the subject of a future communication.

Acknowledgment.—The authors are grateful to Dr. Ross R. Herr for helpful discussions, to Dr. R. W. Rinehart and associates for analyses, to Mr. Dennis T. Weber for titration and to Mr. K. J. Geipel for technical assistance.

(3) B. Bannister and A. D. Argoudelis, *J. Am. Chem. Soc.*, **85**, Jan. 20 (1963).

(4) S. A. Barker, E. J. Bourne, R. Stephens, and D. H. Whiffen, *J. Chem. Soc.*, 4211 (1954).

(5) Melting points, mixed melting points, infrared spectra and X-ray powder patterns.

(6) We are grateful to Dr. Laurens Anderson, Department of Biochemistry, University of Wisconsin, Madison, Wisconsin, for the samples of hexaacetyl-*scyllo*-inosamine and N-acetyl-*scyllo*-inosamine.

(7) L. Anderson and H. A. Lardy, *J. Am. Chem. Soc.*, **72**, 3141 (1950).

(8) H. E. Carter, R. K. Clark, Jr., B. Lytle, and G. E. McCasland, *J. Biol. Chem.*, **175**, 683 (1948).

(9) We are thankful to Dr. George Slomp of The Upjohn Company, Kalamazoo, Michigan, for the interpretation of the n.m.r. spectra.

(10) N.m.r. spectra were observed on a Varian A-60 spectrometer on solutions of the samples in $CDCl_3$. The spectra were calibrated downfield from tetramethylsilane (as zero).

(11) As shown by the n.m.r. spectrum of *n*-butyl carbamate.

RESEARCH LABORATORIES
THE UPJOHN COMPANY — B. BANNISTER
KALAMAZOO, MICHIGAN — A. D. ARGOUDELIS

RECEIVED NOVEMBER 30, 1962

SYNTHESIS AND STRUCTURE OF STEROIDAL 4-PREGNENO[3,2-*c*]PYRAZOLES. A NOVEL CLASS OF POTENT ANTI-INFLAMMATORY STEROIDS

Sir:

It is known that a C-3 oxygen is not required for androgenic, anabolic or progestational activity.[1,2,3] On the other hand, no potent anti-inflammatory agent has been described which lacks a C-3 oxygen.[4] For

(1) See, for instance M. S. de Winter, C. M. Siegman and S. A. Szpilfogel, *Chem. and Ind.*, 905 (1959); Z. Madjerek, J. de Visser, J. Vander and G. A. Overbeek, *Acta Endoc.*, **35**, 8 (1960).

(2) R. O. Clinton, A. J. Manson, F. W. Stonner, R. G. Christiansen, A. L. Beyler, G. O. Potts and A. Arnold, *J. Org. Chem.*, **26**, 279 (1961).

(3) J. A. Zderic, O. Halpern, H. Carpio, A. Ruiz, D. C. Limon, L. Magana, H. Jimenez, A. Bowers and H. J. Ringold, *Chem. and Ind.*, 1625 (1960).

(4) E. W. Boland, *Am. J. Med.*, **31**, 581 (1961). Indeed a C-3 ketone seemed to be required for glucocorticoid activity. The orally active 3-enol