dinitrophenylhydrazone. The nuclear magnetic resonance spectrum[7] (CF_3COOH) shows one OCH_3 (singlet, δ 4.04), one $COCH_3$ (singlet, δ 2.69), broad absorptions at δ 3.27 (2 H), 2.67 (2 H), 7.1–7.9 (3 aromatic H), and a signal at 5.50 (1 H, broad), attributed to a proton on a benzylic carbon bearing a hydroxyl group because of its downfield shift (6.40) on acetylation.[8]

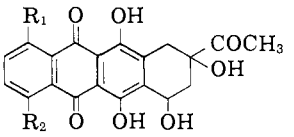
By refluxing with dimethyl sulfate in acetone in the presence of potassium carbonate daunomycinone is converted to a trimethyl ether, $C_{24}H_{24}O_8$, m.p. 193°, four OCH_3, $[\alpha]D$ +181° (*c* 0.1, dioxane), hydroxyl band at 3350 cm.$^{-1}$. This compound shows four sharp singlets at δ 4.00 (6 H, two aromatic OCH_3), 3.89 (3 H, aromatic OCH_3), 3.56 (3 H, aliphatic OCH_3), and 2.40 (3 H, $COCH_3$). A free hydroxyl (singlet, δ 5.02) is clearly recognized by the upfield shift with dilution and downfield shift with acid. A signal at δ 4.92 (1 H, four lines), showing the Ar-CH-O proton, is the X part of an ABX spectrum,[9] the AB part of which consists of two pairs[10] of symmetric doublets centered approximately at 1.87 (1 H) and 2.42 (1 H); a first-order analysis gives J_{AB} = 15 ± 0.2, J_{AX} = 3.5 ± 0.2, and J_{BX} = 2.5 ± 0.2 c.p.s. The magnitude of the J_{AB}, showing geminal coupling, and the shifts of H_A and H_B suggest a methylene β to an aromatic ring. Two doublets (2 H, J = 18.5 ± 0.2 c.p.s.) centered at δ 3.02 and 3.22 (AB pattern) indicate two geminal protons α to the aromatic system, without vicinal hydrogens. A complex multiplet (δ 7–8, 3 H, ABC pattern) suggests three aromatic protons on one ring. This is in agreement with the recovery of salicylic acid by alkaline fusion of either daunomycinone or its trimethyl ether.

The presence of four hydroxyls in daunomycinone is proved by the conversion, on treatment with acetic anhydride and pyridine at 60°, to a tetraacetate $C_{29}H_{26}O_{12}$, one OCH_3, m.p. 225° (from methanol), $[\alpha]D$ −95.5° (*c* 0.11, $CHCl_3$), phenolic (1776 cm.$^{-1}$) and alcoholic (1740 cm.$^{-1}$) acetate bands, no hydroxyl absorption in the infrared.[11] Treatment of daunomycinone with either acids or alkalis gives a bisanhydro derivative, $C_{21}H_{14}O_6$, m.p. 325–330°, conjugated ketone absorption (1685 cm.$^{-1}$), which in turn yields a diacetate, $C_{25}H_{18}O_8$, m.p. 240–243°, one OCH_3, phenolic acetate absorption (1765 cm.$^{-1}$), thus showing the presence of two phenolic and two alcoholic hydroxyls in daunomycinone, the last two being involved in the dehydration reaction.

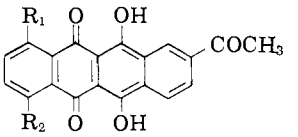
Hydrogenolysis of the benzylic hydroxyl of daunomycinone with Pd on $BaSO_4$ in dioxane affords deoxydaunomycinone, $C_{21}H_{18}O_7$, m.p. 229–231°, $[\alpha]D$ −91° (*c* 0.11, $CHCl_3$), one OCH_3. The n.m.r. spectrum shows OCH_3 (δ 4.05), $COCH_3$ (2.35), two strongly hydrogen-bonded phenolic OH (13.3 and 13.75), one free alcoholic OH (3.75), two benzylic CH_2 (broad, *ca.* 3), CH_2 β to the aromatic system (*ca.* 2), and three aromatic protons (*ca.* 8). Deoxydaunomycinone yields a triacetate, $C_{27}H_{24}O_{10}$ m.p. 126–128°.

Sodium borohydride reduction of daunomycinone, followed by periodate oxidation, affords acetaldehyde, isolated as the 2,4-dinitrophenylhydrazone, in good yield, thus proving the acetyl side chain and its attachment to a hydroxylated carbon atom. Oxidative fission with permanganate of bisanhydrodaunomycinone affords almost quantitatively 1,2,4-benzenetricarboxylic acid (trimellitic acid), m.p. 216–219°, and 3-methoxyphthalic acid, m.p. 168–171°, both identical in all respects with authentic samples.

On the basis of these findings structure I (a or b) and II (a or b), aside from stereochemistry, can be written for daunomycinone and for bisanhydrodaunomycinone, respectively.



Ia, R_1 = H, R_2 = OCH_3
b, R_1 = OCH_3, R_2 = H



IIa, R_1 = H, R_2 = OCH_3
b, R_1 = OCH_3, R_2 = H

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Daunomycin. II. The Structure and Stereochemistry of Daunosamine

Sir:

We wish to present evidence which assigns structure I to daunosamine, the amino sugar moiety of the antitumor antibiotic daunomycin.[1] Daunosamine [hydrochloride (I) $C_6H_{13}O_3N \cdot HCl$,[2] m.p. 168° dec., $[\alpha]D$ at equilibrium −54.5° (H_2O)] is a reducing (positive Fehling, Tollens) amino (positive Elson-Morgan, ninhydrin) trideoxyhexose, yielding ammonia on treatment with hot alkalis. Acetylation of I with acetic anhydride and pyridine gives a crystalline mixture of the anomeric triacetates (II), m.p. 168–170°, $[\alpha]D$ −71° (acetone).

The recovery of malonic dialdehyde, identified by the reaction with thiobarbituric acid,[3] and of acetaldehyde, isolated as the 2,4-dinitrophenylhydrazone, among the products of the periodate oxidation[4] of I indicates the presence of the deoxy group at C-2 (or C-3) and of the methyl group at C-5. The latter is also supported by the fact that I gives a positive iodoform test.

I is readily converted by treatment with 0.3 *N* methanolic HCl to methyl daunosaminide (III), m.p.

(7) N.m.r. spectra were taken with a Varian A60 spectrometer; chemical shifts are in p.p.m. (δ), relative to tetramethylsilane as internal standard.

(8) The spectrum ($CDCl_3$) of the monotrifluoroacetate of daunomycinone, formed in the trifluoroacetic acid solution on standing, shows the same proton at δ 6.55, two strongly hydrogen-bonded hydroxyls (sharp singlets at δ 13.0 and 13.6), and one free OH (broad, *ca.* δ 4.1).

(9) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, p. 132.

(10) One of them is further split by a long-range coupling (*ca.* 1 c.p.s.).

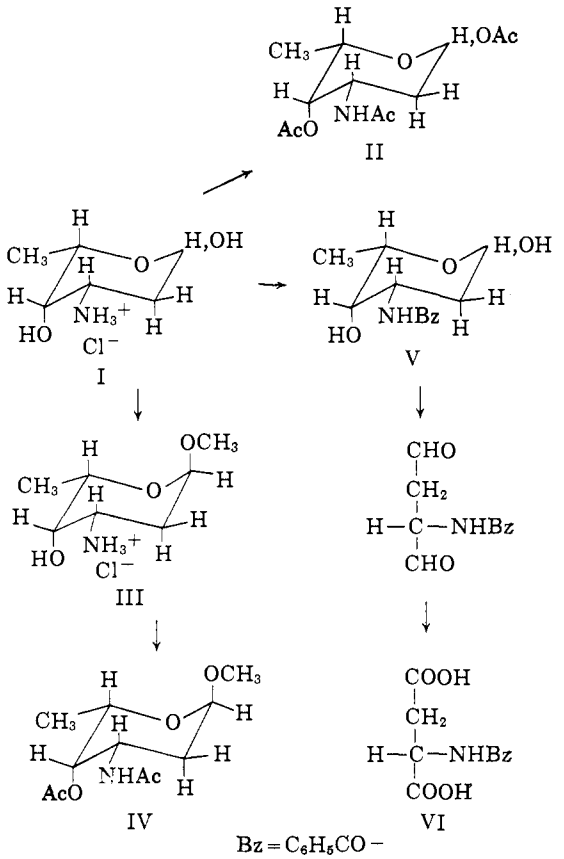
(11) The n.m.r. spectrum confirms the information obtained by the spectra of the other compounds.

(1) F. Arcamone, G. Franceschi, P. Orezzi, G. Cassinelli, W. Barbieri, and R. Mondelli, *J. Am. Chem. Soc.*, **86**, 5334 (1964).

(2) See footnote 3 in ref. 1.

(3) V. S. Waravdecar and L. D. Saslaw, *J. Biol. Chem.*, **234**, 1945 (1959).

(4) R. W. Jeanloz and E. Forchielli, *ibid.*, **188**, 361 (1951).



188–190° dec., $[\alpha]_D$ −130° (H_2O), which gives an N,O-diacetate (IV), m.p. 176–178°, $[\alpha]_D$ −130° ($CHCl_3$), when treated with acetic anhydride and pyridine. III reduces 1 mole of periodate, thus proving the pyranose ring, the deoxy group at C-2, and the attachment of the amino and hydroxyl group at C-3 and C-4. IV is not oxidized by periodate, as expected.

N-Benzoyldaunosamine (V), m.p. 154–156°, $[\alpha]_D$ −107.5° (ethanol), prepared from I with benzoyl chloride and aqueous sodium bicarbonate, reduces 1 mole of periodate yielding acetaldehyde, originated from C-5 and C-6, and a nonvolatile aldehyde (from C-1 to C-4) which is oxidized[5] to N-benzoyl-L-(+)-aspartic acid (VI), $[\alpha]_D$ +33° (H_2O plus 2 equiv. of KOH), identical in all respects with an authentic sample,[6] thus proving the absolute configuration at C-3 as (*S*).[7]

Molecular rotation[8] of I (M_D = −100), almost identical with that of rhodosamine hydrochloride,[9] has been compared with the M_D values of the equilibrium mixtures of the α and β forms of the eight stereoisomeric 2,6-dideoxyhexoses.[10] Only the L-*lyxo* (2-deoxy-L-fucose) and the L-*ribo* (L-digitoxose) compounds show M_D values (−91 and −68, respectively) approaching that of I. However, I cannot have the L-*ribo* configuration because of the spatial arrangement at C-3; therefore the L-*lyxo* configuration is suggested by the optical rotation data.[11]

(5) R. Willstätter and G. Schudel, *Ber.*, **51**, 780 (1918).
(6) E. Fischer, *ibid.*, **32**, 2451 (1899).
(7) R. S. Cahn, C. K. Ingold, and V. Prelog, *Experientia*, **12**, 81 (1956).
(8) $M_D = [\alpha]_D \times M/100$.
(9) H. Brockmann, E. Spohler, and T. Waehneldt, *Ber.*, **96**, 2925 (1963).
(10) T. Reichstein and E. Weiss, *Advan. Carbohydrate Chem.*, **17**, 65 (1962), and references cited therein.
(11) It is generally accepted that replacement of an hydroxyl with an amino group in a pyranoside derivative does not result in a substantial change of the M_D. These observations can be applied successfully also to anomeric mixtures, when the M_D of the α and β forms are not known [see, for instance, E. E. van Tamelen, J. R. Dyer, H. E. Carter, J. V. Pierce, and E. E. Daniels, *J. Am. Chem. Soc.*, **78**, 4817 (1956)].

The nuclear magnetic resonance spectra[12] ($CDCl_3$) of IV shows a triplet at δ 4.75 (H-1),[13] the small splitting of which (in first approximation $J_{1e,2e} = J_{1e,2a} = 2.0$ c.p.s.) indicates the equatorial orientation of the anomeric proton.[14,15] The NH absorption appears as a doublet (J = 8 c.p.s.) at δ 5.85 that disappears with D_2O; a broad adsorption at δ 4.46 is attributed to the H-3, near the amidic proton. The H-4 signal at δ 5.02 is a quartet with splitting 1.5 and 2.5 c.p.s.; the H-5 quartet[16] at δ 4.00 is further split to an octet (J = 1–1.5 c.p.s.) by H-4. The small coupling between H-5, H-4 (*ca.* 1 c.p.s.) and between H-4, H-3 (2.5 c.p.s.) excludes a diaxial orientation[14] between the said protons, whereas the width of the H-3[17] signal suggests an axial orientation of this proton.

The shifts of the acetoxy (δ 2.16) and acetamido (δ 1.91) groups are in good agreement with an axial OAc at C-4 and equatorial NHAc at C-3.[18] Consequently configuration (*S*) has to be assigned to C-4. The methyl group at C-5 cannot be axial (D-configuration), because in this case IV would exist in the more stable C-1 conformation which is not in agreement with the values of the coupling constants.[19]

Stereochemistry of daunosamine is thus established as 3(*S*), 4(*S*), 5(*S*),[7] corresponding to the L-*lyxo* configuration, as shown in structure I.

Acknowledgments.—The authors are grateful to Professor Bruno Camerino for his interest and encouragement.

(12) All spectra were taken with a Varian A60 n.m.r. spectrometer. Chemical shifts are in p.p.m. (δ) relative to tetramethylsilane as internal standard.
(13) The assignment is based on its downfield shift to δ 6.2 in the spectrum of II. This value is in agreement with the findings of R. U. Lemieux and B. Fraser-Reid, *Can. J. Chem.*, **42**, 532 (1964).
(14) R. U. Lemieux, R. K. Kulling, H. J. Bernstein, and W. C. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958).
(15) This is further supported by the shift of the glycosidic OMe (δ 3.31), in agreement with an axial OMe group (see ref. 13). A small amount of the other anomer is present, showing the same signal at δ 3.48.
(16) J = 6.5 c.p.s.; the CH_3 on the same carbon atom appears as a doublet at 1.09, showing the same coupling constants.
(17) The line width after exchange of amidic protons with deuterium, on three-fold treatment with D_2O, measures 18–19 c.p.s. Assuming an 8 c.p.s. average value for $J_{a,a}$ and 2.5 c.p.s. for $J_{a,e}$ and $J_{e,e}$, the calculated width is 13 c.p.s. Assuming an equatorial proton at C-3, the calculated width is 7.5 c.p.s. The signal of C-2 protons does not allow the assignment of the H-3 axial orientation because it is overlapped by the acetyl absorption.
(18) It is well-established[14] that axial acetyl groups absorb at higher values than equatorial ones (axial OAc, δ 2.12–2.25; equatorial OAc and axial NHAc, δ 2.00–2.09; equatorial NHAc, δ 1.90–1.96; see N. Nakajima, A. Hasegawa, and F. W. Lichtenthaler, *Ann.*, **669**, 75 (1963), and references cited therein). In methyl 3-acetamido-2,4-di-O-acetyl-3,6-dideoxy-α-L-taloside, NHAc (equatorial) lies at 1.93 and -OAc (axial) at C-4 lies at 2.18 [A. C. Richardson and K. A. McLauchlan, *J. Chem. Soc.*, 2499 (1962)].
(19) Assignment of the α-glycoside structure to compounds III and IV is based on M_D value of III (−290), which is very near to that calculated (−273) for the unknown methyl 2-deoxy-α-L-fucoside, showing the same configuration. The latter number was obtained by applying a correction of +30 [D. H. Whiffen, *Chem. Ind.* (London), 964 (1956)] to the M_D value of methyl 2-deoxy-α-L-galactoside, the D-isomer of which is known [W. C. Overend, F. Shafizadeh, and M. Stacey, *J. Chem. Soc.*, 671 (1950)].

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