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BUTIROSINS A AND B,[1] AMINOGLYCOSIDE ANTIBIOTICS. II. MASS SPECTROMETRIC STUDY

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The potential usefulness of mass spectrometry in providing structural information of intact aminoglycoside-aminocyclitol antibiotics has been indicated by the preliminary results of a study involving paromomycin and related compounds.[2] We now wish to report on the actual use of this technique in elucidating the structural details of butirosins A (Ia) and B (Ib),[1] novel members of this clinically important class of antibiotics, during the course of their structural elucidation.

The mass spectral studies were carried out with poly-N-acetyl-poly-O-trimethylsilyl[2] (N-Ac-O-TMS)-butirosins A and B (IIa and b), with the aid of a deuterioacetyl analog, N-Ac-d_3-O-TMS-butirosin A (IIIa).[3] As indicated previously, the structural units of Ia and Ib had been shown to be neosamine C (Nc),[4] deoxystreptamine (D),[4] (S)-(-)-4-amino-2-hydroxybutyric acid (Ab),[4] and a pentose (Pn)[4] (D-xylose in Ia and D-ribose in Ib), by largely chemical studies.[1]

A small peak at m/e 1227 (0.03%, 0.2%)[5] (1239 from IIIa, 0.8%) and a stronger one at 1212 (0.9%, 6.3%) (1224 from IIIa, 12%) may be assigned as the molecular-ion (M^+) peak and the ($M - CH_3$) peak, respectively. A mol wt of 1227 would indicate the presence of only one each of the four structural units (Nc, D, Ab, and Pn)[4] in the molecule, linked together in such a manner as to contain seven O-TMS and four N-Ac groups, as exemplified by formulas IIa and b. Furthermore, based on the reasonable assumption that Nc and Pn, like other hexoses and pentoses, exist in ring form, a mol wt of 1227 would indicate that the four structural units are linked together in an acyclic manner, as exemplified by formulas IIa and b.

A peak at m/e 389 (19%, 37%) (395 from IIIa, 44%) corresponds to the Nc fragment, resonance-stabilized,[2] originating as a terminal, glycosidic (rather than aglycone or ether) unit carrying two O-TMS and two N-Ac groups.[6] This assignment was corroborated by a peak at

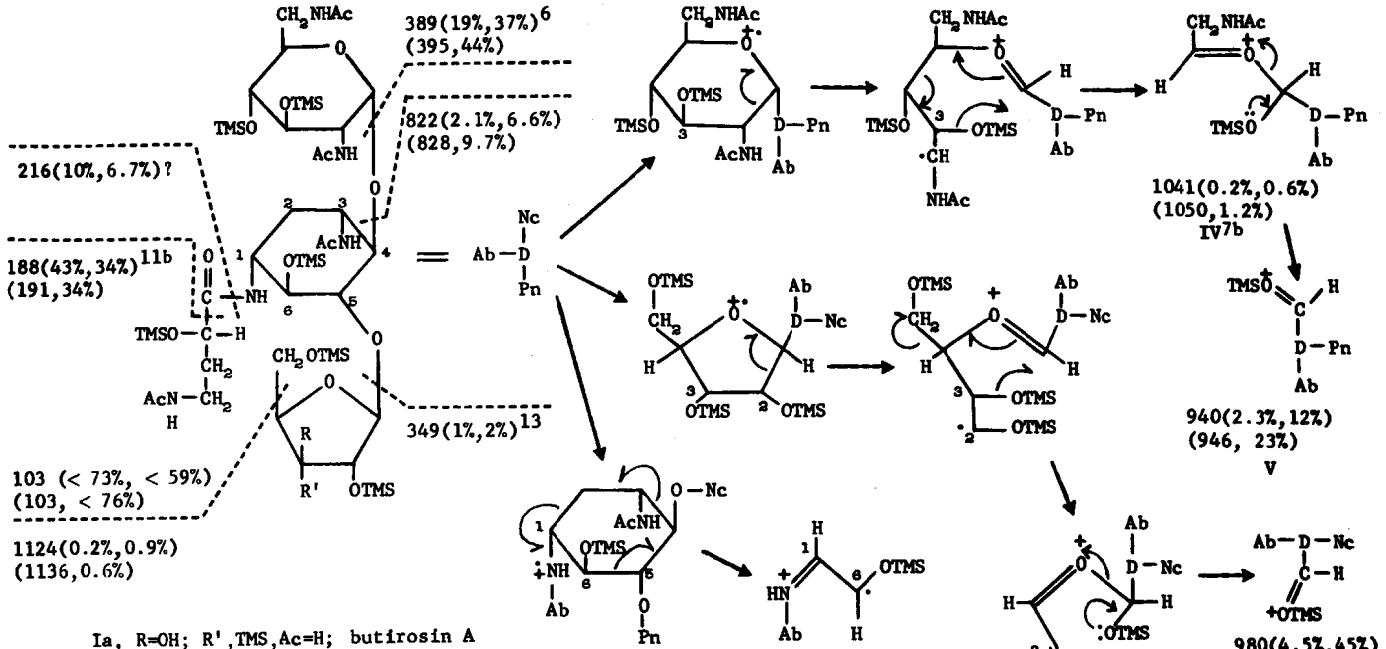

Figure 1. Significant fragments from electron-impact fragmentations of IIa, IIb, and IIIa, with numbers designating m/e values from IIa and b (intensities[5b] from IIa, from IIb) and (m/e values, intensities, from IIIa). "Nc," "D," "Ab," and "Pn" represent the four structural units (cf. text related to ref 4); TMS = trimethylsilyl ($(CH_3)_3Si$—); Ac = acetyl or deuterioacetyl.

822 (2.1%, 6.6%) (828 from IIIa, 9.7%), corresponding to the loss of this terminal unit and its glycosidic oxygen from M^+ (M — 389 — 16), and a peak at 940 (2.3%, 12%) (946 from IIIa, 23%), which corresponds to a rearranged fragment V, consisting of Ab, D, Pn, and the anomeric carbon of Nc which carries an O-TMS group originating from C-3 of Nc.[7,8,9,10]

A peak at 188 (43%, 34%) (191 from IIIa, 34%) corresponds to a $TMSO-\overset{+}{C}H-CH_2-CH_2NHAc$ fragment from Ab,[11] indicating that Ab is a terminal unit (amide or ester) carrying one O-TMS and one N-Ac group.

A peak at 980 (4.5%, 45%) (992 from IIIa, 53%) may be assigned to a rearranged fragment VI, consisting of Ab, D, Nc, and the anomeric carbon of Pn which carries an O-TMS group originating probably from C-3 of Pn.[12] This assignment requires that Pn exists as a terminal unit (glycosidic) carrying three O-TMS groups.[13]

If three of the four structural units, Nc, Ab, and Pn, are each a terminal unit attached to the rest of the molecule through one bond, as indicated above, then they cannot be attached to each other but must be individually attached to the fourth structural unit D. Such an arrangement is exemplified in formula Ia and b.

The presence of the carboxyl group of Ab as an amide, as in Ia and Ib, is indicated by the presence of a strong infrared peak at 1650 cm^{-1} and the absence of other carbonyl stretching absorption above 1700 cm^{-1}. The attachment of Ab as amide instead of ester, together with the presence of one O-TMS and one N-Ac group (calculated from data above) in the D moiety, requires the attachment of Nc and Pn to D as O-glycosides instead of N-glycosides.

A small peak at m/e 346 (2.0%, 2.1%) (349 from IIIa, < 3.7%) could be partially accounted for by fragment VII,[14] which would suggest the attachment of Ab and TMS at the C-1 amino and C-6 hydroxy grouping of D, hence the attachment of Nc and Pn at the C-4 and C-5 (or C-5 and C-4) oxygens of D, as shown in IIa and b. However, the possibility of contribution from other interfering ions to this peak precludes a definite assignment.[15]

Cleavage of the C-4-C-5 bond of Pn, if a furanoside as indicated in IIa and b, would contribute to the peaks at m/e 103 (73%, 59%) (76% from IIIa) and 1124 (0.2%, 0.9%) (1136 from IIIa, 0.6%). However, the former could have originated also from a pentopyranoside;[9c,16] the latter, though more specific,[9c] also may have some contribution from other nonspecific ions.[15]

In summary, mass spectral study has provided crucial evidence in establishing the molecular formulas of butirosins A and B ($C_{21}H_{41}N_5O_{12}$) and has shown that Ab, Nc, and Pn are individu-

ally attached, as amide and O-glycosides, to the D moiety. Other details of attachment, pertaining to the exact linkage location on D, ring size, and anomeric configuration, will be shown in the following communication.[17]

References and Footnotes

1. P. W. K. Woo, H. W. Dion, and Q. R. Bartz, Tetrahedron Lett., preceding paper.
2. D. C. DeJongh, J. D. Hribar, S. Hanessian, and P. W. K. Woo, J. Am. Chem. Soc., 89, 3364 (1967).
3. The mass spectra were obtained by Dr. Alan Duffield with an AEI MS-9 spectrometer using the direct inlet system and an ionization potential of 70 ev.
4. The abbreviations "Nc", "D", "Ab", and "Pn" will be used to designate either the structural units or their derivatives, either as individual compounds or as components of molecules, whichever is appropriate according to context.
5. (a) The intensities of the same peak from IIa and IIb will be represented by, respectively, the first and second % values in parentheses, without specific designation. (b) The height of the peak at m/e 217 ($(CH_3)_3SiO\text{-}CH{=}CH\text{-}CH{=}\overset{+}{O}Si(CH_3)_3$ from the pentose moiety (9a)) is used as reference (100%) in calculating relative intensities of other peaks. The strongest peak, however, is the $Si(CH_3)_3^+$ peak at 73 (>195%, 256%) (237% from IIIa). (c) The relatively low intensities of the peaks in the high m/e region of the spectrum of IIa, compared to those of IIb and IIIa, are undoubtedly due to variations in the instrument conditions used.
6. Further confirmation of this assignment is provided by a peak at m/e 299 (8.1%, 8.0%), corresponding to the loss of TMSOH from the m/e 389 fragment, and a peak at 240 (23%, 28%), corresponding to the loss of CH_3CONH_2 from the m/e 299 fragment. These relationships are confirmed by metastable ions (m*) at 230 and 192.3. The corresponding peaks from IIIa are found at 305 (7.7%) and 243 (30%), accompanied by m* at 235.5.
7. (a) This fragment is analogous to fragment "C_8", $CH_3O\text{-}\overset{+}{C}H\text{-}OCH_3$, described by K. Heyns, et al. (8), and to the "J_1^1" fragment, $(CH_3)_3Si\text{-}\overset{+}{O}{=}CHOR$, described by O. S. Chizhov, et al. (9a,c). (b) The presence of fragment IV (precursor of V) is mildly suggested by a peak at 1041 (0.2%, 0.6%) (1050 from IIIa, 1.2%).
8. K. Heyns, G. Kiessling, and D. Müller, Carbohyd. Res., 4, 452 (1967).
9. (a) O. S. Chizhov, N. V. Molodtsov, and N. K. Kochetkov, ibid., 4, 273 (1967); (b) N. K. Kochetkov, O. S. Chizhov, and N. V. Molodtsov, Tetrahedron, 24, 5587 (1968); (c) N. K. Kochetkov and O. S. Chizhov, ibid., 21, 2029 (1965).
10. For other examples of the migratory aptitude of the O-TMS group see J.-Å. Gustafsson, R. Ryhage, J. Sjövall, and R. M. Moriarty, J. Am. Chem. Soc., 91, 1234 (1969), and references therein.
11. (a) This assignment is confirmed by the presence of a strong peak at m/e 188 from the N-Ac-O-TMS derivative of N^1-(4-amino-2-hydroxybutyryl)-2-deoxystreptamine (17) and by the absence of such a peak from the N-Ac-O-TMS derivatives of paromomycin II, neamine, and related compounds (2). (b) Loss of CH_3CONH_2 from this fragment contributes to the peak at 129 (64%, 62%) (60% from IIIa), as confirmed by a weak m* at 88.5. (c) A peak at 216 (10%, 6.7%) (219 from IIIa masked by isotopic clusters from 217) may possibly be assigned to the Ab fragment.
12. (a) Fragment VI is analogous to the "J_1^1" fragment, $RO\text{-}CH{=}\overset{+}{O}CH_3$ (R = CH_3, CD_3), from methyl 2,3,5-tri-O-methyl-α(and β)-L-arabofuranoside and its various O-deuteriomethyl analogs described by Kochetkov and Chizhov (9c). (b) A similar fragment, $TMSO\text{-}CH{=}\overset{+}{O}CH_3$, is indicated by a peak at m/e 133 from methyl 2,3,5-tri-O-(trimethylsilyl)-β-D-ribofuranoside. (c) The mechanism of formation, proposed for VI in Fig. 1, should be applicable to the analogous fragments mentioned in 12a and 12b immediately above. It differs from that suggested by S. Hanessian, D. C. DeJongh, and J. A. McCloskey [Biochim. Biophys. Acta, 117, 480 (1966)] for a slightly analogous fragment, "B-$\overset{+}{C}H(OH)$," from cordecipin.
13. A peak at m/e 259, corresponding to the loss of TMSOH from the Pn fragment (calcd m/e, 349), is present (49%, 36%) (42% from IIIa).
14. (a) The N-Ac-O-TMS derivative of N^1-(4-amino-2-hydroxybutyryl)-2-deoxystreptamine (11a, 17) also gives this peak. (b) The N-acetyl-O-methyl analog of VII, $Ac\overset{+}{N}H{=}CH\text{-}\dot{C}H\text{-}OCH_3$, m/e 115, is given by N,N'-diacetyl-6-O-methyl-2-deoxystreptamine but not N,N'-diacetyl-5-O-methyl-2-deoxystreptamine.
15. This conclusion is based on mass spectral comparison with related compounds (11a, 2).
16. A peak at m/e 103, corresponding to $CH_2{=}\overset{+}{O}TMS$, is obtained from the tri-O-TMS derivatives of both methyl β-D-ribofuranoside and methyl β-D-ribopyranoside.
17. P. W. K. Woo, H. W. Dion, and Q. R. Bartz, Tetrahedron Lett., following paper.