M^{-1} s^{-1}, of which there are several, ^{57}Fe NMR may provide a convenient method of studying electron transfer reactions.

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Biosynthetic Studies on Validamycins: A $C_2 + C_2 + C_3$ Pathway to an Aliphatic C_7N Unit[1]

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Validamycin A[3] is the major component of the validamycin complex, used in the Orient to treat sheath blight disease in rice. The discovery of validamycins, followed by the isolation of the antibacterial pseudo-α-galactopyranose,[4] introduced novel (hydroxymethyl)cyclitols including validamine, hydroxyvalidamine,[5] valienamine, valiolamine,[6] and carbocyclic analogues of hexopyranoses (pseudosugars).[7] The valienamine and hydroxyvalidamine units have also been identified as building blocks of recently discovered microbial α-glucosidase inhibitors[8] (Chart I).

Nothing has been reported previously about the biosynthesis of these (hydroxymethyl)cyclitols. In principle, validamine, hydroxyvalidamine, valienamine, and valiolamine units could be considered aliphatic analogues of the "*m*-C_7N" units,[9] widely found in quinonoid antibiotics and related compounds. However, we present evidence here derived from feeding ^{13}C-labeled precursors to *Streptomyces hygroscopicus* var. *limoneus*[10] indicating that both the validamine and valienamine units are, on the contrary, biosynthesized by a pathway involving a seven-carbon sugar which is formed by a C_2-group transfer ($C_2 + C_2 + C_3$) related to the pentose phosphate pathway.[11]

Chart I. Structures of Validamycin A and (Hydroxymethyl)cyclitols

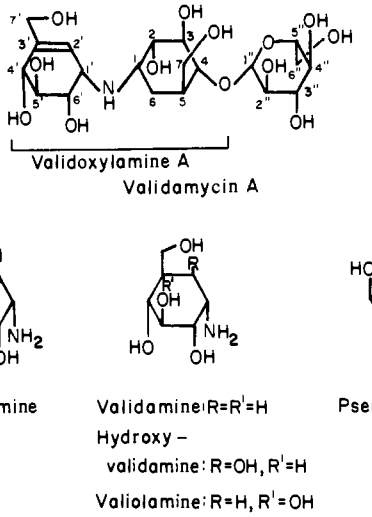
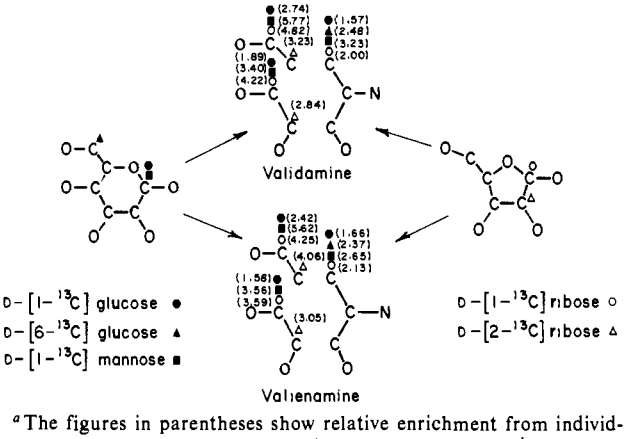


Table I. ^{13}C NMR Signals[a] for the Validoxylamine A Unit of Validamycin A Derived from *D*-[U-^{13}C]Glucose

C-*n*	δ, ppm[b]	pattern	J_{C-C}, Hz	changes in C-*n* on irradiating C-*m*	C-*m*
C-1	56.2	t + d + s	38.5, 35.5	→ d + s	C-6
C-2	~75.5	*c*			
C-3	75.2	d + s	37.6	→ s	C-4
C-4	86.8	d + s	37.6		
C-5	39.9	d + s	37.4		
C-6	29.4	d + s	35.5		
C-7	64.3	d + s	37.4	→ s	C-5
C-1′	54.9	t + d + s	42.3, 39.5	→ d + s	C-2′
C-2′	125.5	d + s	42.3		
C-3′	141.7	d + s	47.5		
C-4′	74.0	d + s	41.1		
C-5′	~76.0	*c*			
C-6′	72.0	d + s	39.5		
C-7′	64.1	d + s	47.5	→ s	C-3′

[a] Spectra were recorded in deuterium oxide on an NSF-250 spectrometer. [b] See ref 10. [c] Due to overlap of these signals, the splitting patterns could not be determined.

Scheme I. Labeling of C_7 Units by Carbohydrate Precursors[a,b]



[a] The figures in parentheses show relative enrichment from individual precursors (see Table III, supplementary material). [b] Precursors were added to the production media, which contain 1% D-glucose, after 24-h incubation (see ref 10).

The *m*-C_7N units of geldanamycin[9b,12a] and pactamycin[9b,12b] are constructed from C_4 and C_3 units and the cyclopentanoid unit of pactamycin[13] from C_6 and C_1 units, and analogous mechanisms

(1) Presented in part at Antibiotics 86, Granada, Spain, June 19-24, 1986; Abstr., pp 36-37.
(2) On leave of absence, 1981-1983, from the Institute of Antibiotics, Chinese Academy of Medical Science, Beijing.
(3) (a) Iwasa, T.; Yamamoto, H.; Shibata, M. *J. Antibiot.* **1970**, *23*, 595-602. (b) Ogawa, S.; Nose, T.; Ogawa, T.; Toyokuni, T.; Iwasawa, Y.; Suami, T. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2369-2374.
(4) Miller, T. W.; Arison, B. H.; Albers-Schonberg, G. *Biotechnol. Bioeng.* **1973**, *15*, 1075-1080. According to the "IUPAC-IUB Tentative Cyclitol Nomenclature Rules" (*J. Biol. Chem.* **1968**, *243*, 5809-5819), (+)-(1,2/3,4,5)-5-(hydroxymethyl)-1,2,3,4-cyclohexanetetrol.
(5) Hydroxyvalidamine is a building unit of validamycin B, the less biologically active component of the validamycin complex. In validamycin B the validamine unit of validamycin A is replaced by the hydroxyvalidamine unit (Chart I): Horii, S.; Iwasa, T.; Kameda, Y. *J. Antibiot.* **1971**, *24*, 57-63.
(6) Valiolamine is a building unit of recently isolated validamycin G. In validamycin G the validamine unit of validamycin A is replaced by the valiolamine unit (Chart I): (a) Kameda, Y.; Asano, N.; Yoshikawa, M.; Takeuchi, M.; Yamaguchi, T.; Katsui, M.; Horii, S.; Fukase, H. *J. Antibiot.* **1984**, *37*, 1301-1307. (b) Kameda, Y.; Asano, N.; Yamaguchi, T.; Matsui, K.; Horii, S.; Fukase, H. *J. Antibiot.* **1986**, *34*, 1491-1494.
(7) The term "pseudosugar" was coined to designate 5-(hydroxymethyl)-1,2,3,4-cyclohexanetetrol: McCasland, G. E.; Furuta, S. *J. Org. Chem.* **1966**, *31*, 1516-1521. See also: Ogawa, S.; Ara, M.; Kondoh, T.; Saitoh, M.; Masuda, R.; Toyokuni, T.; Suami, T. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 1121-1136.
(8) A Review: Truscheit, E.; Frommer, W.; Junge, B.; Muller, L.; Schmidt, D. D.; Wingender, W. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 744-761.
(9) (a) Rinehart, K. L., Jr.; Shield, L. S. *Fortschr. Chem. Org. Naturst.* **1976**, *33*, 231-307. (b) Rinehart, K. L., Jr.; Potgieter, M.; Jin, W.-Z.; Pearce, C. J.; Wright, D. A.; Wright, J. L. C.; Walter, J. A.; McInnes, A. G. In *Proceedings of the International Conference on Trends in Antibiotic Research*; Umezawa, H., Demain, A. L., Hata, T., Hutchinson, C. R., Eds.; Japan Antibiotics Research Association: Tokyo, 1982; pp 171-184.
(10) For the fermentation conditions and ^{13}C NMR assignment of validamycin A, see: Jin, W.-Z.; Rinehart, K. L., Jr.; Toyokuni, T. *J. Antibiot.* **1987**, *40*, 329-339.
(11) Comprehensive texts: (a) Horecker, B. L. *Pentose Metabolism in Bacteria*; Wiley: New York, 1962. (b) Wood, T. *The Pentose Phosphate Pathway*; Academic: New York, 1985.
(12) (a) Rinehart, K. L., Jr.; Potgieter, M.; Wright, D. A. *J. Am. Chem. Soc.* **1982**, *104*, 2649-2655. (b) Rinehart, K. L., Jr.; Potgieter, M.; Delaware, D. L.; Seto, H. *J. Am. Chem. Soc.* **1981**, *103*, 2099-2101.

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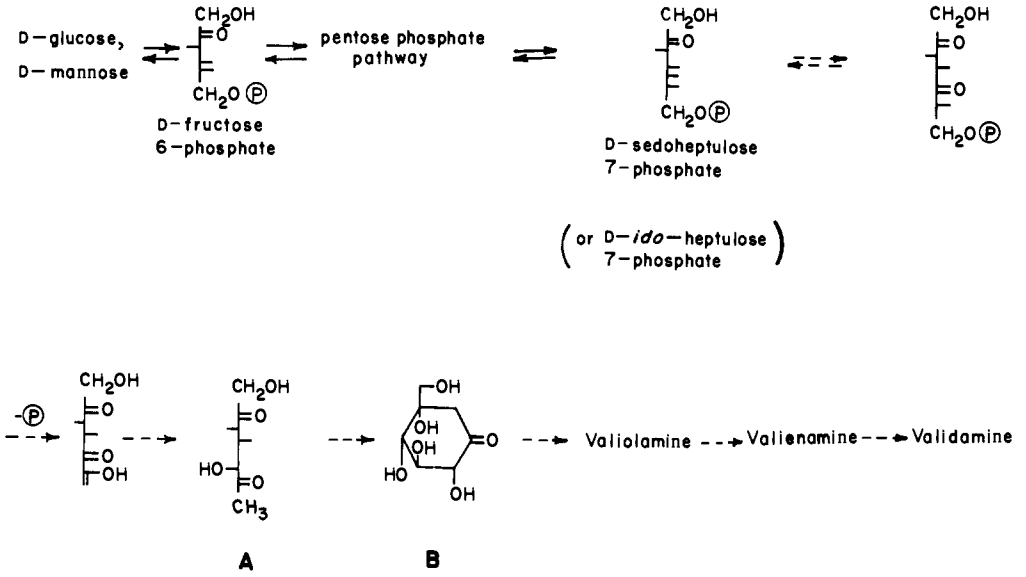
Scheme II. Proposed Biosynthetic Pathway to the Aliphatic *m*-C_7N Units



were initially investigated for validamine and valienamine. The ($C_6 + C_1$) pathway was effectively eliminated by a low incorporation of methionine (0.0026%), while poor incorporations of glycerate and glycolate (glycerate, 0.0079%; glycolate, 0.0012%) cast doubt on the ($C_4 + C_3$) possibility, since both precursors had labeled well the *m*-C_7N unit of geldanamycin.[14] On the other hand, better incorporation of D-ribose (0.57%) than D-glucose (0.353%) suggested a pathway similar to that by which *C*-methylcyclitols are biosynthesized ($C_5 + C_2$).[15]

Administration of D-[$^{13}C_6$]glucose followed by interpretation of the ^{13}C–^{13}C labeling patterns of the resulting validamycin A clearly revealed that both seven-carbon skeletons were constructed by a new combination of $C_2 + C_2 + C_3$ (Table I). The splitting pattern indicated that only C-1 and C-1′ were strongly coupled to two other carbons. Although the signals for C-2 and C-5′ overlapped each other, the coupling constants determined the coupling of C-2 to C-1 and C-4′ to C-5′.

The feeding of D-[1-^{13}C]-[16] and D-[6-^{13}C]glucose, D-[1-^{13}C]-mannose,[16] and D-[1-^{13}C]- and D-[2-^{13}C]ribose also demonstrated a labeling distribution (Scheme I) different from that of a shikimate-related pathway, suggesting that glucose, mannose, and ribose underwent cleavage between C-2 and C-3 (nonoxidative pentose phosphate pathway)[17] and that the C_3 fragment was derived from D-glyceraldehyde 3-phosphate[18] through the glycolytic pathway.

Considering the stereochemistry of validamine and valienamine and the known cyclization pathway to 5-dehydroquinate,[19] either D-sedoheptulose 7-phosphate[20] or D-*ido*-heptulose 7-phosphate could be a close precursor of the cyclic initial intermediates.[21] The pathway shown in Scheme II seems plausible, since oxidation of the hydroxyl group on C-5 of the heptulose might facilitate the elimination of orthophosphate.[22] An intramolecular aldol condensation between C-2 and C-7 of the 2,6-diketose A would yield (hydroxymethyl)cyclohexanone B, presumably the key cyclic intermediate[23] to the validamine and valienamine units.

Note Added in Proof. Conclusions similar to ours have independently been reached very recently for the valienamine unit of acarbose.[24]

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Registry No. Validamycin A, 37248-47-8; validamine, 32780-32-8; valienamine, 38231-86-6.

Supplementary Material Available: Table II, incorporation of labeled precursors into validamycin A, and Table III, ^{13}C enrichment of validamycin A carbons by labeled precursors (2 pages). Ordering information is given on any current masthead page.

(13) Potgieter, M. Ph.D. Dissertation, University of Illinois, Urbana-Champaign, IL, 1983. Reference 9b.

(14) Haber, A.; Johnson, R. D.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 3541–3544.

(15) Woeber, G.; Hoffmann-Ostenhof, O. *Eur. J. Biochem.* **1970**, *17*, 393–396.

(16) D-[1-^{13}C]Glucose (90.7 atom % ^{13}C) and D-[1-^{13}C]mannose (90.3 atom % ^{13}C) were synthesized from D-arabinose and sodium [^{13}C]cyanide according to the procedure employed by Serianni et al.: Serianni, A. S.; Nunez, H. A.; Barker, R. *Carbohydr. Res.* **1979**, *72*, 71–78.

(17) The same labeling distribution from D-[1-^{13}C]mannose[16] as from D-[1-^{13}C]glucose suggests the intermediary of fructose 6-phosphate (Table III, supplementary material).[11]

(18) The introduction of C-1 of glucose into C-6 of validamine and C-2′ of valienamine, both of which were also labeled by C-6 of glucose, agreed with the rapid interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate by triose phosphate isomerase.

(19) Srinivasan, P. R.; Rothschild, J.; Sprinson, D. B. *J. Biol. Chem.* **1963**, *238*, 3176–3182. Bohm, B. A. *Chem. Rev.* **1965**, *65*, 435–466.

(20) In the pentose phosphate pathway, transketolase catalyzes the transfer of the C_2 fragment from xylulose 5-phosphate and fructose 6-phosphate to ribose 5-phosphate, producing sedoheptulose 7-phosphate. The pathway converting ribose to xylulose 5-phosphate via ribulose 5-phosphate is known.[11]

(21) If D-*ido*-heptulose 7-phosphate rather than D-sedoheptulose 7-phosphate is a real precursor, D-xylose 5-phosphate could reasonably be expected to form D-*ido*-heptulose 7-phosphate by a C_2 addition. In some plants it has been found that the feeding of D-xylose results in the accumulation of D-*ido*-heptulose: Rendig, V. V.; McComb, E. A. *Arch. Biochem. Biophys.* **1962**, *99*, 409–413.

(22) Eliminations of orthophosphate from phosphate esters of 3-hydroxy aldehydes and of 3-hydroxy esters have been reported. See, for example: Brown, D. M.; Fried, M.; Todd, A. R. *J. Chem. Soc.* **1955**, 2206–2210. Riley, G.; Turnbull, J. H.; Wilson, W. *J. Chem. Soc.* **1957**, 1373–1379.

(23) Support of this cyclic intermediate has been provided by the isolation of valiolamine, probably formed by transamination of B, from the fermentation broth of the validamycin-producing organism. See ref 6a.

(24) Floss, H. G.; Keller, P. J.; Beale, J. M. *J. Nat. Prod.* **1986**, *49*, 957–970.