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STUDIES ON THE BIOSYNTHESIS OF TERRECYCLIC ACID A II[1)]

CONFIRMATION OF THE CYCLIZATION MECHANISM AND HYDROGEN SHIFTS USING [2-$^{2}H_3$]ACETATE AND [2-$^{13}C^{2}H_3$]ACETATE

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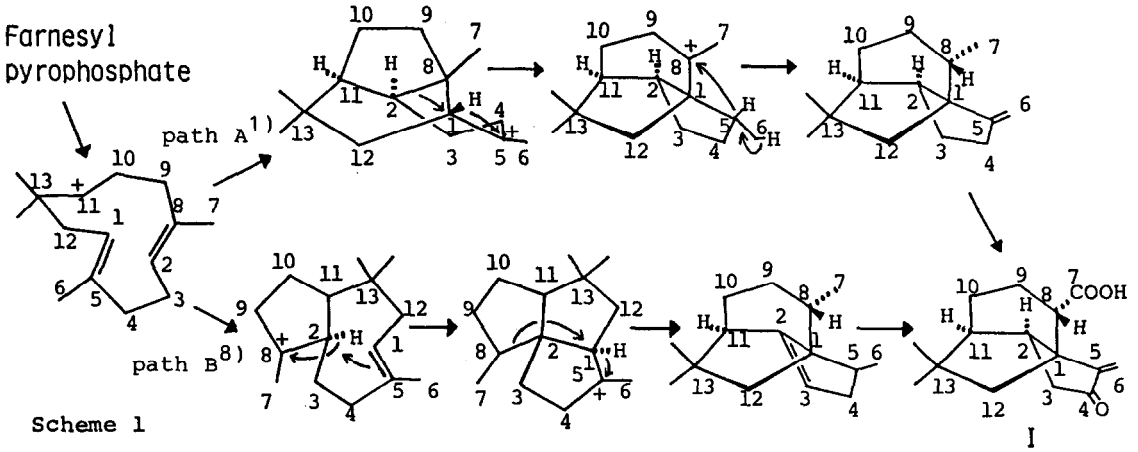
Abstract: Feeding experiments with [2-$^{3}H_3$]acetate and [2-$^{13}C^{2}H_3$]acetate in Aspergillus terreus Thom No.14 indicated that the hydrogen at C-2 in terrecyclic acid A is incorporated without migration from the precursor acetic acid; the results favour our group's earlier speculation for the cyclization to the tricyclic skeleton in the biosynthetic scheme.

Terrecyclic acid A (I) is a sesquiterpene antitumor antibiotic produced by Aspergillus terreus Thom No.14[2,3)] with a novel carbon skeleton, common to an antitumor substance quadrone (II).[4,5)] Asp. terreus No.14 produces II and several structurally related sesquiterpenes.[6,7)] It is natural that the compounds with such a novel carbon skeleton became an attractive target for biosynthetic studies. The biosyntheses of I and II have been independently studied using [1-^{13}C], [2-^{13}C] and [1,2-$^{13}C_2$]acetate by our group[1)] and Brown University's group.[8)]

The incorporation patterns of these labelled acetates into I and II by the two groups were identical each other and the results from these feeding experiments demonstrated that I and II were biosynthesized by way of an isoprene pathway.[9)] The speculative biosynthetic pathways of the two groups, however, were not identical (Scheme 1). In our pathway (path A), firstly the C-2 - C-11 and the C-1 - C-8 bonds are formed, and then C-2 - C-8 bond shift to C-2 - C-1 with migration of the proton at C-1 to C-5. On the other hand in the path B (Brown University's group), firstly the formation of C-2 and C-11 is followed by the linkage of C-1 and C-2 after migration of the



Scheme 1

proton at C-2 to C-8, and then finally C-8 - C-2 bond shifts to C-8 - C-1.

These two pathways could be distinguished by determining whether the proton at C-2 has been retained as the acetate unit (path A) or has migrated from another carbon (path B). Therefore we performed the feeding experiments of [2-$^{2}H_3$]acetate and [2-$^{13}C^{2}H_3$]acetate in Asp. terreus No.14 in order to distinguish these postulated pathways.

The suspension of the washed mycelium of Asp. terreus No.14 was supplemented with [2-$^{2}H_3$] sodium acetate and incubated for 3 days. I was isolated from this fermentation broth and converted to dihydroterrecyclic acid A (III).[6] The ^{1}H- and ^{2}H-irradiated ^{13}C-NMR spectrum of III thus obtained[10] (Fig.1) indicates that the incorporation ratio of labelled acetate is very high and that the signals assignable to C-2, 6, 9, 11, 14 and 15 are accompanied by minor signals due to α-deuterium substituted shift. Similarly the signals of C-1, 3, 5, 8, 10 and 13 show small accompanied signals due to β-deuterium substituted shift. These α- and β-substituted shifts are summarized in Table 1 and compatible with the reference values.[11,12]

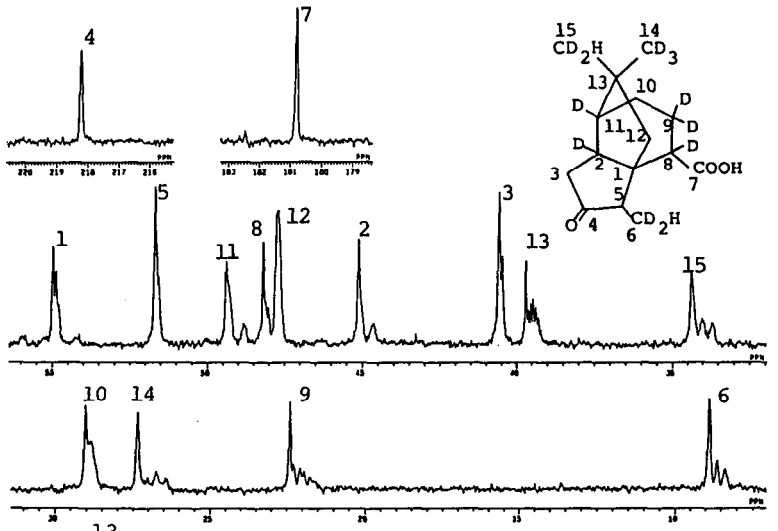


Fig.1 ^{13}C-NMR spectrum of III obtained from the feeding experiment with [2-$^{2}H_3$] sodium acetate.

Table 1 Alpha- and beta-deuterium substituted shifts in ^{13}C-NMR spectra of III. (ppm)

Carbon	III from feeding experiment with [2-$^{3}H_3$]acetate				III from feeding experiment with [2-$^{13}C^{2}H_3$]acetate	
	α		β		α	
C-1			β-1 0.102	β-2 0.190		
C-5			β-1 0.131			
C-11	α-1 0.554				α-1 0.489	
C-8			β-1 0.088	β-2 0.175		
C-2	α-1 0.408				α-1 0.423	
C-3			β-1 0.116			
C-13			β-1 0.073	β-2 0.161		
			β-3 0.234	β-4 0.331		
			β-5 0.429	β-6 0.496		
C-15	α-1 0.350	α-2 0.656			α-1 0.321	α-2 0.635
C-10			β-1 0.131	β-2 0.175		
C-14		α-2 0.613			α-1 0.299	α-2 0.606
	α-3 0.905				α-3 0.912	
C-9	α-1 0.336	α-2 0.657	β-1 0.132		α-1 0.321	α-2 0.657
C-6	α-1 0.263	α-2 0.511			α-1 0.263	α-2 0.511

These data clearly indicated that deuterium(s) from $^{2}H_3$-acetate were incorporated at C-2, 9 and 11 together with at C-6, 14 and 15 methyls. Furthermore the splitting patterns of C-9 and C-1 due to β-deuterium substitution suggested that a deuterium was incorporated also at C-8 although the α-substituted signal corresponding to C-8 can not be observed because of the overlapping of the signal of C-12 carbon. This conclusion was supported by the fact that ^{2}H-NMR spectrum of III obtained by the above feeding experiment (Fig.2) shows a signal due to two deuteriums between 2.5 ppm and 3.0 ppm.

The presence of the deuterium at C-8 could be interpreted as a result of the migration of the deuterium from C-1 (path A) or from C-2 (path B), because the C-8 carbon was originated from the carboxyl carbon of an acetate unit.

To clarify whether the deuterium at C-2 had been retained from acetate

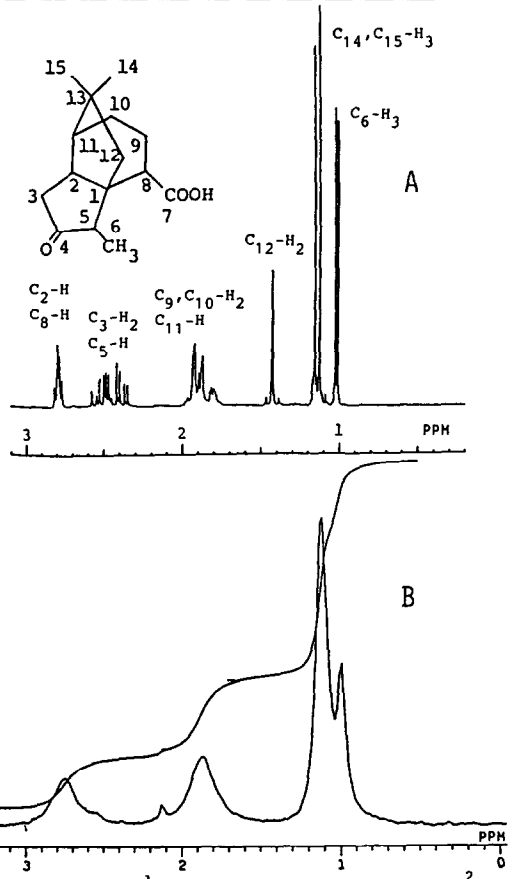


Fig.2 A; 400MHz ^{1}H-NMR spectrum of III. B; 60MHz ^{2}H-NMR spectrum of III obtained from the feeding experiment with [2-$^{2}H_3$] sodium acetate.

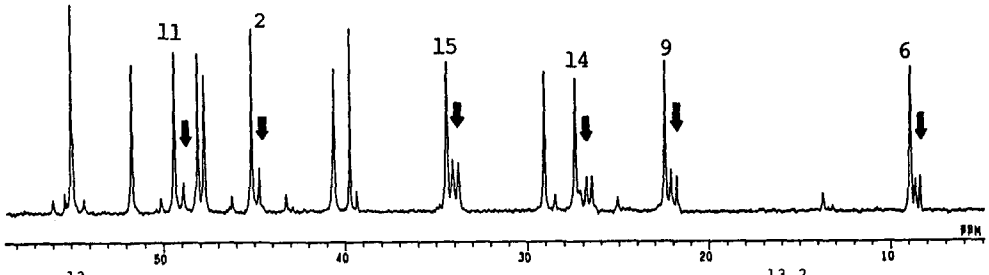


Fig.3 ^{13}C-NMR spectrum of III obtained from the feeding experiment with [2-$^{13}C^{2}H_3$] sodium acetate (5-58ppm). The arrow indicates signals due to α-substituted shift.

or migrated from other carbon, [2-$^{13}C^{2}H_3$]acetate feeding experiment was performed. Diluted with four fold cold sodium acetate, [2-$^{13}C^{2}H_3$]acetate[13] was added to the fermentation broth of Asp. terreus No.14 at 48hr and the fermentation was continued for an additional 48hr. I from this fermentation was also reduced to III. The ^{13}C-NMR spectrum of labelled III (Fig.3) shows clearly that the C-2 carbon along with C-6, 9, 11, 14 and 15 carbons are accompanied by α-deuterium substituted peaks. This confirmed that the deuterium at C-2 carbon was retained from the acetate unit in the biosynthetic pathway of I. Therefore, the hydrogen at C-8 should be transferred from C-1 probably via C-5. Accordingly, these results support our speculation (path A) and exclude the path B for the cyclization scheme. Further studies on the biosynthesis of I are in progress.

References and notes

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