Tetrahedron Letters No. 48, pp 4875 - 4878, 1972. Pergamon Press. Printed in Great Britain.

ON THE BIOLOGICAL ORIGIN OF THE NITROAROMATIC UNIT OF THE ANTIBIOTIC AUREOTINE

R.Cardillo, C.Fuganti, D.Ghiringhelli, D.Giangrasso, P.Grasselli

Istituto di Chimica del Politecnico, Centro del CNR per la Chimica delle Sostanze Organiche Naturali, 20133 Milano, Italy

(Received in UK 11 October 1972; accepted for publication 26 October 1972)

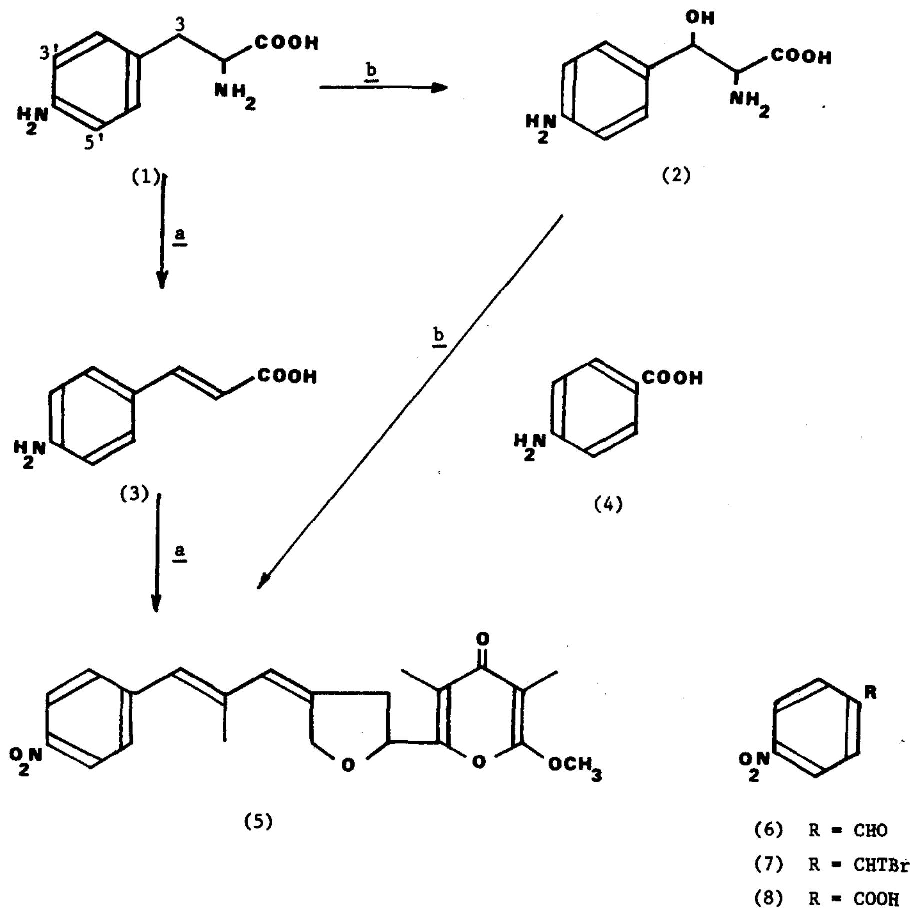
The recent report[1] on feeding experiments with ^{13}C labelled precursors supporting the large derivation of the antibiotic aureotine (5) from propionate prompted us to present our results on the work designed to establish the biological origin of the nitroaromatic, C_6-C_1 unit of (5).

Since the observation[2] that side-chain labelled p-aminophenylalanine (1) is incorporated into the C_6-C_3 framework of chloramphenicol in Streptomyces sp. 3022a, it seemed possible that the same amino acid (1) could be the precursor of the nitroaromatic unit occuring in aureotine (5), produced by Streptomyces thioluteus,[3] through a degradation process proceeding along paths a or b.

We now report on feeding experiments which support this view, and, further, define some of the operations of path b.

Tritium labels were inserted ortho to the amino group of D,L-p-aminophenylalanine (1) by acid-catalysed exchange with tritiated water. The labelling pattern of the radioactive (1) was determined by iodination with ICl in diluited HCl to 3',5'-diiodo-4'-aminophenylalanine which retains less than 15% of the starting tritium activity.[4]

$[3\text{-}^{3}H]$p-aminophenylalanine (1) was obtained as follows. p-Nitrobenzaldehyde (6) was reduced with sodium borotritiide to the ^{3}H-labelled alcohol, which was converted into the bromoderivative (7) in quantitative yield. Bromide displacement with an



excess of sodio-acetamidomalonate in dimethylformamide at 50° led to a product which, after hydrolysis-decarboxylation and hydrogenation, yielded the required $[3-^{3}H]$p-aminophenylalanine (1) without significant ^{3}H loss, in 65% overall yield.

The two samples were mixed and crystallised to constant activity to afford $[3,3'-5'-^{3}H_{3}]$p-aminophenylalanine (1). The ratio of the labels between the positions <u>ortho</u> to the aromatic amino group and position 3 was determined by iodination and resulted as being 1:2.4.

[3',5'-$^{3}H_2$]p-aminocinnamic acid (3) was obtained by exchange with tritiated water, and, similarly, [3,5-$^{3}H_2$]p-aminobenzoic acid (4). The same procedure could not be used for labelling erythro-p-aminophenylserine (2), owing to the instability of the product under the acidic treatment. Therefore, ^{3}H labels were inserted into the aromatic ring of 2-amino-4-nitrotoluene by acid catalysed exchange with tritiated water. The activating amino group was removed by diazotisation in ethanol, followed by treatment with Cu powder at 70°, to give ^{3}H-p-nitrotoluene. The latter upon oxidation with CrO_3 in acetic anhydride, gave the diacetale of p-nitrobenzaldehyde. Hydrolysis of the protecting groups yielded p-nitrobenzaldehyde (6), which retains nearly all the starting ^{3}H activity. Oxidation of radioactive (6) to p-nitrobenzoic acid (8) caused, as expected, no ^{3}H loss. From [ring-^{3}H] p-nitrobenzaldehyde (6), D,L-erythro[ring-^{3}H] p-aminophenylserine (2) was obtained according to the reported procedure[5].

The aureotine (5) isolated in feeding experiments in *Streptomyces thioluteus* with the abovementioned labelled precursors (1), (2), (3) and (4) was purified by chromatography and crystallised to constant activity. Its labelling pattern was determined by degradation[3] to p-nitrobenzaldehyde (6) and p-nitrobenzoic acid (8) upon ozonolysis.

TABLE

Relative ^{3}H molar activities and (incorporations%)

Expt.	Precursors	Aureotine (5)	$p-NO_2C_6H_4CHO$ (6)	$p-NO_2C_6H_4COOH$ (8)
1	D,L-[3,3',5'-$^{3}H_3$] p-aminophenylalanine (1)	100 (12)	97	94
2	D,L-erythro[ring-^{3}H]p-aminophenylserine	100 (25)	105	101
3	[3',5'-$^{3}H_2$]p-aminocinnamic acid (3)	no incorp.		
4	[3',5'-$^{3}H_2$]p-aminobenzoic acid (4)	($<$ 0.01)		

The relative ^{3}H molar activities (Table) and the incorporation values show that p-aminophenylalanine (1) and erythro-p-aminophenylserine (2), but not p-aminocinnamic acid (3), are on the biosynthetic way to aureotine (5). Although direct evidence that the carbon atom at C-3 of (1) and (2) is retained at C-1 in the nitroaromatic C_6-C_1 unit of aureotine (5) is still lacking, the relative activities for p-nitrobenzaldehyde (6) and p-nitrobenzoic acid (8) obtained in Expt.1 with doubly labelled (1) indicate that, if this does occur, complete removal of the ^{3}H originally present at C-3 of (1) takes place at some stage of the biosynthesis.

The evidence therefore suggests that the C_6-C_3 unit of p-aminophenylalanine (1) is degraded *via* p-aminophenylserine (2) to the nitroaromatic unit of aureotine (5) with loss of the hydrogen originally present in benzylic position, while p-aminocinnamic acid (3) seems outside the metabolic pathway. Furthermore, p-aminobenzoic acid (4) appears to be incorporated very poorly.

We thank professor R.Locci, Università di Milano, for a strain of I.P.V. 2050. The help of professors B.Camerino, A.Grein and C.Spalla, Istituto di Ricerche Farmitalia, Milano, in preliminary feeding experiments with *Streptomyces distallicus* is greatly acknowledged.

REFERENCES

[1] M.Yamazaki, F.Katoh, J.Ohishi, and Y.Koyama, *Tetrahedron Letters*,2701,1972

[2] R.McGrath, L.C.Vining, F.Sala, D.W.S.Westlake, *Can.J.Biochem.*,*46*,587,1968

[3] Y.Hirata, H.Nakata, K.Yamada, K.Okuhara, and T.Naito, *Tetrahedron*,*14*,252,1961

[4] P.Block,Jr., *J.Org.Chem.*,*21*,1237,1956

[5] R.Weichert, *Arkiv Kemi*,*25*,231,1966