Communications to the editor

THE IDENTIFICATION OF 3-AMINO-5-HYDROXYBENZOIC ACID AS A NEW NATURAL AROMATIC AMINO ACID

Sir:

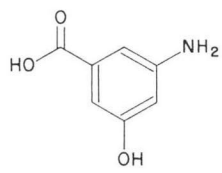
The existence of a new natural aromatic amino acid, 3-amino-5-hydroxybenzoic acid (**1**), was initially predicted by us[1] from analysis of the structures of antibiotics of the ansamycin and maytansinoid group.[2] We then showed that labelled 3-amino-5-hydroxybenzoic acid was efficiently and specifically incorporated by *Streptomyces* cultures into the aminoquinonoid ring and adjacent C-8 of the ansamycin antibiotic actamycin (**2**),[1] and also into the 4a-amino-6-methylbenzoquinone nucleus of porfiromycin (**3**)[3], a member of the mitomycin group of antibiotics.[4] These results established the amino acid (**1**) as a key intermediate in the biosynthesis of these two important classes of antibiotics. GHISALBA *et al.*[5] subsequently confirmed the involvement of 3-amino-5-hydroxybenzoic acid in ansamycin biosynthesis by mutant studies with the rifamycin-producing *Nocardia mediterranei*. Structural considerations indicated a possible role for the same species in the formation of other types of *Streptomycete* antibiotics.[1] The most obvious example is that of ferrimycin A_1,[6] in which the unmodified aromatic nucleus of the amino acid (**1**) is attached through its carboxyl and amino groups to the rest of the complex antibiotic by amide and acylated *gem*-diamino linkages, respectively.

We have now shown that growing cultures of *S. verticillatus* not only utilise exogenous 3-amino-5-hydroxybenzoic acid (**1**) for the biosynthesis of mitomycins[3], but also produce and excrete the free amino acid into the culture medium.

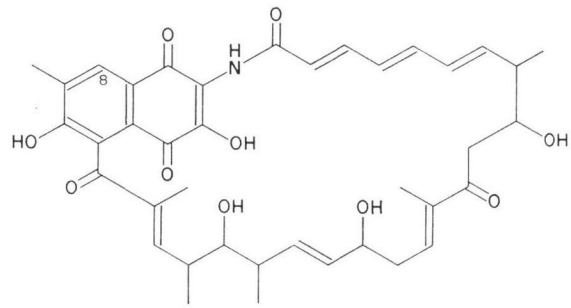
S. verticillatus (Lederle Laboratories strain AB-929) was grown in shaken culture on a complex medium (meat extract, yeast extract, peptone, soybean meal, glucose, sodium chloride, and calcium carbonate, adjusted to pH 7.3~7.5). High performance liquid chromatography of the culture filtrate after 96 hours growth indicated the presence of a low level of a component with retention time similar to that of 3-amino-5-hydroxybenzoic acid (**1**), which was not detectable in the unfermented culture medium. However, the complexity of the mixture precluded an unequivocal identification of this component by h.p.l.c. Conventional amino acid analysis could not be used, since the detector systems rely on the colour developed with ninhydrin reagent, which does not react with the present amino acid (**1**). Accordingly, isotope dilution analysis was used to verify the presence of endogenous 3-amino-5-hydroxybenzoic acid in the fermentation.

The culture liquor analysed was that from which [6-methyl-^{13}C]-porfiromycin had been extracted after pulse feeding [carboxy-^{13}C]-3-amino-5-hydroxybenzoic acid hydrochloride (85 atom% excess ^{13}C in carboxyl carbon) over a growth period of 124 hours[3]. An aliquot was freeze-dried, and the residue acetylated (Ac_2O - Et_3N, 20°C, 18 hours). This reaction mixture was evaporated, and the residue extracted with hot ethyl acetate. The extract was methylated (CH_2N_2, Et_2O, 0°C, 5 minutes) and then chromatographed on a layer of silica gel (EtOAc - hexane, 1: 1). The zone with Rf value similar to that of authentic methyl 3-acetoxy-5-(acetylamino)-benzoate (**4**) was eluted and subjected to coupled gas-liquid chromatography/mass spectrometry (2% OV-17, temperature programmed 140~290°C at 8°C/minute). The component with the same retention time as authentic ester (**4**) showed *m/z* (percentage of base peak) 252 (8) and 251 (7); 221 (5) and 220 (4); 210 (59) and 209 (46); 178 (8) and 177 (9); 168 (100) and 167 (84); 137 (16) and 136 (15); and 109 (46). A sample of ester (**4**) of natural isotope composition showed *m/z* 252 (1) and 251 (7), (M); 220 (4), (M−OMe); 210 (6) and 209 (55), (M−CH_2CO); 178 (8), (M−OMe−CH_2CO); 168 (10) and 167 (100), (M−$2CH_2CO$); 136 (13), (M−$2CH_2CO$−OMe); 109 (34), (167-CO_2CH_2). Comparison of the isotope ratios in these two mass spectra shows that the labelled ester (**4**) isolated from the fermentation contains 52 atom% excess of ^{13}C over natural abundance in the carbonyl group of the ester.

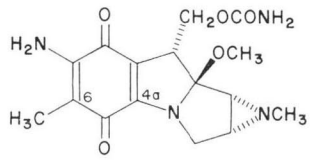
The observed reduction in ^{13}C content of the carboxyl group of the amino acid (**1**) from 85 to 52 atom % excess over natural abundance during



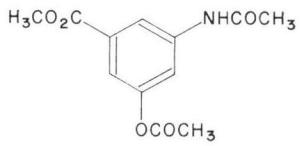
(1)



(2)



(3)



(4)

the course of the fermentation results from dilution with unlabelled material produced by *S. verticillatus*, and establishes 3-amino-5-hydroxybenzoic acid (**1**) as a new, naturally-occurring aromatic amino acid.

The known occurrences of ansamycin[2], mitomycin[4] and ferrimycin[6] antibiotics indicate that formation of 3-amino-5-hydroxybenzoic acid is relatively common amongst microorganisms of the genus *Streptomyces*, and occurs also in the genera *Micromonospora* and *Nocardia*. The various isolations of maytansinoids[2] confirm the microbial genus *Nocardia* as a source of this amino acid and extend the range of probable sources to higher plants of the families *Celastraceae* and *Rhamnaceae*.* 3-Amino-5-hydroxybenzoic acid is probably derived from glucose *via* an early intermediate of the shikimic acid pathway[3]. The widespread operation in microorganisms and higher plants of this major metabolic route to aromatic compounds[7] suggests a wider occurrence of 3-amino-5-hydroxybenzoic acid (**1**) than is yet recognised.

Acknowledgements

We thank Dr. J. R. D. McCormick, Lederle Laboratories, American Cyanamid Company, for a culture of *S. verticillatus* AB-929, fermentation of which was carried out by Mrs. M. G. Anderson and Mrs. J. M. Rothschild.

* Or microorganisms associated with these higher plants[8].

Jeffrey J. Kibby
Rodney W. Rickards

Research School of Chemistry
Australian National University
P. O. Box 4, Canberra, A. C. T. 2600
Australia

(Received February 13, 1981)

References

1) Kibby, J.J.; I.A. McDonald & R.W. Rickards: 3-Amino-5-hydroxybenzoic acid as a key intermediate in ansamycin and maytansinoid biosynthesis. J. Chem. Soc., Chem. Comm. 1980: 768~769, 1980

2) Wehrli, W.: Ansamycins. Chemistry, biosynthesis and biological activity. Top. Curr. Chem. 72: 21~49, 1977
Rinehart, K. L., Jr. & L. S. Shield: Chemistry of the ansamycin antibiotics. Fortschr. Chem. Org. Naturst. 33: 231~307, 1976
Kupchan, S. M.; Y. Komoda, A. R. Branfman, A. T. Sneden, W. A. Court, G. J. Thomas, H. P. J. Hintz, R. M. Smith, A. Karim, G. A. Howie, A. K. Verma, Y. Nagao, R. G. Dailey, Jr., V. A. Zimmerly & W. C. Sumner, Jr.: The

maytansinoids. Isolation, structural elucidation, and chemical interrelation of novel ansa macrolides. J. Org. Chem. 42: 2349~2357, 1977

3) ANDERSON, M. G.; J. J. KIBBY, R. W. RICKARDS & J. M. ROTHSCHILD: Biosynthesis of the mitomycin antibiotics from 3-amino-5-hydroxybenzoic acid. J. Chem. Soc., Chem. Comm. 1980: 1277~1278, 1980

4) FRANCK, R. W.: The mitomycin antibiotics. Fortschr. Chem. Org. Naturst. 38: 1~45, 1979

5) GHISALBA, O.; H. FUHRER, W. J. RICHTER & S. MOSS: A genetic approach to the biosynthesis of the rifamycin-chromophore in *Nocardia mediterranei*. III. Isolation and identification of an early aromatic ansamycin-precursor containing the seven-carbon amino starter-unit and three initial acetate/propionate-units of the ansa chain. J. Antibiotics 34: 58~63, 1981
GHISALBA, O. & J. NÜESCH: A genetic approach to the biosynthesis of the rifamycin-chromophore in *Nocardia mediterranei*. IV. Identification of 3-amino-5-hydroxybenzoic acid as a direct precursor of the seven-carbon amino starter-unit. J. Antibiotics 34: 64~71, 1981

6) BICKEL, H.; P. MERTENS, V. PRELOG, J. SEIBL & A. WALSER: Stoffwechselprodukte von Mikroorganismen. 53. Ueber die Konstitution von Ferrimycin A_1. Tetrahedron Suppl. 8: 171~179, 1966

7) HASLAM, E.: The Shikimate Pathway. Wiley, New York, 1974
FLOSS, H. G.: The shikimate pathway. pp. 59~89, *in* T. SWAIN, J. B. HARBORNE & C. F. VAN SUMERE (*eds*), Biochemistry of Plant Phenolics. Plenum, New York, 1979

8) BRYAN, R. F.; C. J. GILMORE & R. C. HALTIWANGER: Crystal and molecular structure and absolute configuration of maytansine 3-bromopropyl ether. J. Chem. Soc. Perkin II 1973: 897~902, 1973