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FURTHER EXAMPLES OF BIOLOGICAL C-METHYLATION: NOVOBIOCIN AND ACTINOMYCIN

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AMONG major biological pathways leading to compounds carrying C-methyl groups are those involving terpenoid intermediates (e.g. mevalonic acid[1]), branched chain amino-acids (e.g., valine, leucine, isoleucine[2]), propionic acid (or methylmalonyl-coenzyme-A[3,4]), or transmethylation from C_1-donor systems (e.g., methionine,

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[1] cf. J.W.Cornforth, J.Lipid Res. 1, 3 (1959).

[2] cf. W.E.Knox and E.J.Behrman, Ann.Rev.Biochem. 28, 223 (1959).

[3] A.J.Birch, E.Pride, R.W.Rickards, P.J.Thomson, J.D.Dutcher, D.Perlman and C.Djerassi, Chem.and Ind. 1245 (1960).

[4] J.W.Corcoran, T.Kaneda and J.C.Butte, J.Biol.Chem. 235, PC29 (1960); H.Grisebach, H.Achenbach and U.C.Grisebach, Naturwiss. 47, 206 (1960); Z.Vanek, personal communication.

choline, betaine). This last process has been shown to be responsible for "introduced"methyl substituents in acetate-derived aliphatic[5,6] and phenolic[7] structures, terpenoids,[8] branched chain monosaccharides,[9] and the porphyrin-like moiety of vitamin B_{12}.[10] We now wish to report the occurrence of a further type of biological transmethylation.

Fermentation of Streptomyces niveus in the presence of L-[$^{14}CH_3$]methionine yielded novobiocin[11] (I, * denotes ^{14}C) (with 10% isotope incorporation) in which the

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5 A.J.Birch, P.Fitton, E.Pride, A.J.Ryan, H.Smith and W.B.Whalley, J.Chem.Soc. 4576 (1958).

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8 G.J.Alexander and E.Schwenk, J.Amer.Chem.Soc. 79, 4554 (1957).

9 Z.Vanek, personal communication.

10 R.Bray and D.Shemin, Biochim.Biophys.Acta 30, 647 (1958).

11 J.W.Hinman, E.L.Caron and H.Hoeksema, J.Amer.Chem.Soc. 78, 2019 (1956); idem, ibid. 79, 3789 (1957); C.H.Shunk, C.H.Stammer, E.A.Kaczka, E.Walton, C.F.Spencer, A.N.Wilson, J.W.Richter, F.W.Holly and K.Folkers, ibid. 78, 1770 (1956).

radioactivity was distributed specifically between the C-methyl of the coumarin system (35%), the O-methyl of the noviose moiety (35%), and the gem-dimethyl group of the latter (31%, probably one methyl only being introduced). The same methyl cation donor was utilised (to the extent of 15%) in the biosynthesis of actinomycin (a typical structure[12] II) by an unidentified *Streptomyces* species, providing specifically the two nuclear C-methyl groups (28% of the total antibiotic radioactivity), in addition to portion, presumably the N-methyl groups, of the peptide chains.

These results provide the first unequivocal demonstration of methyl transfer from methionine (probably as the *S*-adenosyl derivative[13]) to carbon in an aromatic ring system derived by the shikimic acid route,[14] since it has been shown[15] that the coumarin moiety of novobiocin (I) arises from tyrosine.

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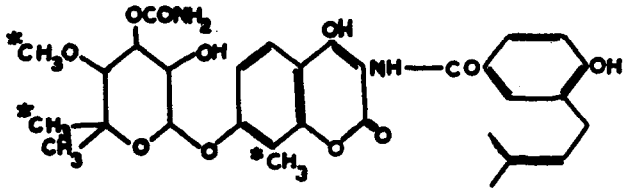
[12] H.Brockmann, *Fortschr.Chem.org.Naturstoffe*, Springer, Vienna, **18**, 1 (1960).

[13] L.W.Parks, *J.Amer.Chem.Soc.* **80**, 2023 (1958).

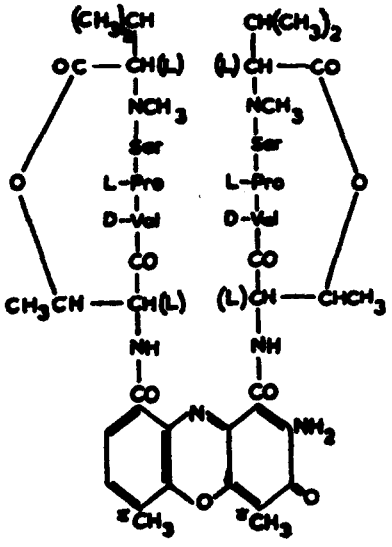
[14] B.D.Davis, *Arch.Biochem.Biophys.* **78**, 497 (1958), and references there cited.

[15] K.Chambers, G.W.Kenner, M.J.T.Robinson and B.R.Webster, *Proc.Chem.Soc.* 291 (1960).

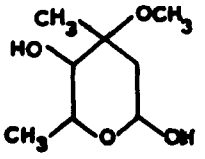
It is also likely, but not proven, that the nucleus of the actinomycins (e.g. II) is formed by oxidative dimerisation of appropriate hydroxy-anthraniloyl units (cf. ref.[16]) arising from shikimic acid.



I



II



III

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[16] H.Brockmann and H.Lackner, Naturwiss. 47, 230 (1960).

It is notable that the three "introduced" methyl substituents of novobiocin carry approximately the same radioactivity (cf. terramycin[6] and mycophenolic acid[7]). We have not identified the variable peptide chains (cf. ref.[12]) present in the actinomycin preparation, but assuming the presence of four N-methyl groups in terminal N-methyl valine and sarcosine units (as in the commonest natural actinomycins[12]), then the extent of isotope utilisation for nuclear C-methylation is comparable to, although slightly lower than, that for N-methylation. The derivation from methionine of a C-methyl group of noviose is in accord with similar results[9] for the related cladinose (III) of erythromycin, and indicates formation of such branched chain monosaccharides by methylation of a pre-existing skeleton, as opposed to elaboration by aldol- or ketol-type condensations of small units[17] or by processes similar to those of fatty acid biosynthesis.[18]

The production by mutation of Streptomyces strains

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[17] L.Hough and J.K.N.Jones, Nature 167, 180 (1951).

[18] R.B.Woodward, Festschrift Arthur Stoll, Birkhauser, Basle, p.524 (1957).

capable of producing demethyltetracyclines[19] by omission of the biological C-methylation stage,[6] indicates that such demethyl antibiotics could probably be obtained by similar mutations in the organisms producing novobiocin or actinomycin.

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[19] J.R.D.McCormick, N.O.Sjolander, U.Hirsch, E.R.Jensen and A.P.Doerschuk, J.Amer.Chem.Soc. 79, 4561 (1957).