Comparison of 6-FSA Properties

	Isolated	Authentic
m.p.	134°	134–137° (134° mixed)
$FeCl_3$ Test	red	red
*R_f (Uvl. Color)	(*a*) 0·60 (yel)	(*a*) 0·60 (yel)
	(*b*) 0·82 (bl)	(*b*) 0·82 (bl)
Diazo Spray	Light brown	Light brown
2,4-dnp m.p.	265°	272° (265° mixed)
*R_f 2,4-dnp	0·75	0·75

* BuOH–0·5 *N* NH_4OH–EtOH 70/20/10

Thus, we envision that aromatization takes place first either from glucose or from activated acetate to give 6-methylsalicylic acid (I), which in turn by a two step oxidation at the methyl group becomes 6-formylsalicylic acid (II). The finding of 3-hydroxyphthalic acid (VII) in filtrates and in replacement media is taken simply as a result of further oxidation of (II) at the formyl group, which yields an additional carboxyl. The hypothetical 5-hydroxy-6-formylsalicylic acid (III) is postulated to arise by nuclear hydroxylation[13] of (II). Although this intermediate has yet to be detected, its structure is not unlike the 'orsellinic units' found in the lichen depsides[14]. Decarboxylation of (III) affords gentisaldehyde (VIII) directly, whereas oxidative decarboxylation would give rise to gentisic acid (IV). The mechanism of the conversion of (II) to the gentisic series by way of (III) is subject to experimental examination with isotopic carbon, since in each of the foregoing pathways a different carboxyl can be lost.

It is not yet clear whether gentisaldehyde or gentisic acid is closer to the open chain 'pre-patulin' (V or Va). From the replacement experiments it would appear that gentisic acid is more directly related to patulin (VI) than is the aldehyde, as might well be expected from the fact that the acid and patulin have the same empirical formula. Certainly, however, BIRKENSHAW's attractive hypothesis[6] or some variation thereof seems at this point to have promise of explaining the actual mechanism of this interconversion.

The effects of trace metals in altering the ratios of metabolic products are probably exerted at the following points: either the two step oxidation of (I) to (II), blocking of which can result in the accumulation of 6-methylsalicylate in the medium, or at the oxidative *vs* straight decarboxylation of (III), which results in the primary accumulation of either gentisaldehyde or of gentisic acid. The accumulation of the last mentioned metabolites under low iron concentration may be related to the finding that the analogous homogentisic acid oxidase of animal tissues[15] is an iron requiring enzyme. Reduction of gentisaldehyde by glycolytic fragments or enzymatic dismutation of this compound will produce gentisyl alcohol (IX), which was originally found as a metabolite in *P. patulum*[16].

The second group of compounds, pyrogallol (XIII), *p*-hydroxybenzoic acid (XII), and anthranilic acid are more closely allied to the well characterized components of the aromatic amino acid pathway from shikimic acid[17]. Accordingly, we hypothesize that pyrogallol may arise from shikimate via gallic acid (XI). The latter can be conceived of coming either by some direct aromatization of shikimic acid, or more likely from oxidation of *p*-hydroxybenzoate. Indeed, preliminary evidence by paper chromatographic and color tests indicated that gallate and shikimate can be found in the broth of strain 2159A, and the conversion of shikimic acid to *p*-hydroxybenzoate in replacements has already been mentioned.

In this connection, it must be pointed out that BIRCH *et al.*[18], working with the related *P. griseofulvum*, conclude that 6-methylsalicylic acid arises from the direct 'head to tail' condensation of acetate. This is to a measure borne out by our experiments with acetate in replacement media. However the acetate hypothesis cannot be directly invoked to explain pyrogallol formation since condensation followed by cyclization of C_2 units results in meta orientated polyphenolic compounds. Nor does the conversion to pyrogallol from any of the products related to 6-methylsalicylic acid appear any the more likely. An outside alternative is that pyrogallol is formed from nuclear hydroxylation of resorcinol. The possibility of such ortho oxidations in nature has been considered by SESHADRI[14] in a discussion of the C_8 lichen substances. A search for the presence of resorcinol or for meta hydroxylated acids in *P. patulum* has failed to uncover evidence for this type of substance.

We are faced therefore with the consideration that within the same microorganism two pathways toward aromatization might co-exist: the one from hexose to shikimic acid and thence to pyrogallol, *p*-hydroxybenzoic and the aromatic amino acids, and the other from hexose, possibly through acetate to 6-methylsalicylic acid and to patulin. If both pathways are present we might also expect to find that there are one or more points of convergence. We are planning to resolve some of these questions by means of mutant, isotopic tracer, and enzymological techniques.

Zusammenfassung

Ausser Patulin, Gentisinsäure und 6-Methylsalicylsäure wurden die folgenden Verbindungen als Metaboliten von *P. patulum* Stamm 2159A gefunden: 6-Formylsalicylsäure, 3-Oxyphthalsäure, Pyrogallol, *p*-Oxybenzoesäure und Anthranilsäure. Eine aliphatische Vorstufe von Patulin und eine Substanz vom Depsidtypus konnten noch nicht näher identifiziert werden. Die möglichen Stoffwechselzusammenhänge zwischen diesen Verbindungen wurden diskutiert.

[13] J. H. WEISBURGER, E. K. WEISBURGER, and H. P. MORRIS, Science *125*, 503 (1957).

[14] T. R. SESHADRI, Exper. Suppl. II, 258 (1955).

[15] D. I. CRANDALL, *A Symposium on Amino Acid Metabolism* (Baltimore 1955), p. 867.

[16] J. H. BIRKENSHAW, A. BRACKEN, S. A. MICHAEL, and H. RAISTRICK, Lancet *245*, 625 (1943). – A. BRACK, Helv. chim. Acta *30*, 1 (1947).

[17] B. D. DAVIS, *A Symposium on Amino Acid Metabolism* (Baltimore 1955), p. 799. – D. B. SPRINSON, *A Symposium on Amino Acid Metabolism* (Baltimore 1955), p. 817.

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