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INCORPORATION OF SHIKIMIC ACID INTO 1,2-DIHYDROXY-ANTHRAQUINONE (ALIZARIN) BY <u>RUBIA</u> <u>TINCTORUM</u> L.

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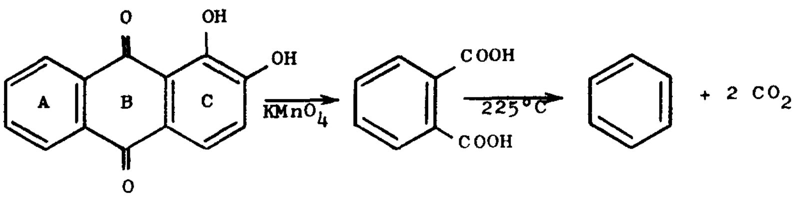
The biosynthetic pathway of anthraquinone formation in fungi has been mainly established by Gatenbeck (see ref.1), who demonstrated that anthraquinones are formed in these organisms entirely from acetate. The mechanism of their formation should involve the condensation of 7 molecules of acetyl- or malonyl-CoA. This mechanism has also been implied in the formation of anthraquinone in higher plants[1], however no experimental evidence exists so far.

This possibility has now been tested experimentally for higher plants by feeding acetate-2-^{14}C to the root system of <u>Rubia</u> <u>tinctorum</u>. Alizarin was isolated, found to be radioactive and purified to constant specific activity. The incorporation of acetate was about o,oo69 %. Degradation of the alizarin molecule (I) showed surprisingly that radioactivity was restricted to ring B and C while ring A remained unlabelled. Uniformly labelled phenylalanine was not incorporated into alizarin when supplied to the madder roots. However, when the incorporation experiments were performed with DL 1,6-^{14}C-shikimic acid an incorporation of o,774 % based on the natural D-isomer of shikimic acid was achieved. The undiluted alizarin from the root had a specific activity of

$2{,}6 \times 10^6$ dpm/mMole. Degradation of the alizarin molecule (I) to phthalic acid (II) and decarboxylation of II to benzene (III) and CO_2 showed that the entire radioactivity was confined to the ring A benzene nucleus of the molecule.

I II III



spec.act. dpm/mMole	$2{,}56 \times 10^4$	$2{,}54 \times 10^4$	$2{,}54 \times 10^4$	0

Furthermore shikimic acid is incorporated in toto since experiments with uniformly labelled shikimic acid demonstrated that the radioactivity of the carboxyl group of this precursor is found in the carboxyl groups of II.

These results indicate that either higher plants or at least Rubiaceae, have developed an entirely different pathway for the biosynthesis of anthraquinones than fungi, or that in the experiments on the biosynthesis of fungal anthraquinones[2] acetate was incorporated via shikimic acid; this critizism has also been raised for fungal naphtoquinone synthesis[3].

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REFERENCES

1 R.H. Thomson, in "Chemistry and biochemistry of plant pigments" (T.W.Goodwin, ed.), p 309, Academic Press, New York (1965)

2 S.Gatenbeck, Acta Chem. Scand. **14**, 296 (1960)

3 G.B. Cox and F.Gibson, Biochem.J. **100**, 1 (1966)