Stereochemistry of Hydrogen Elimination from C-6 of Shikimate in Naphthoquinone Biosynthesis

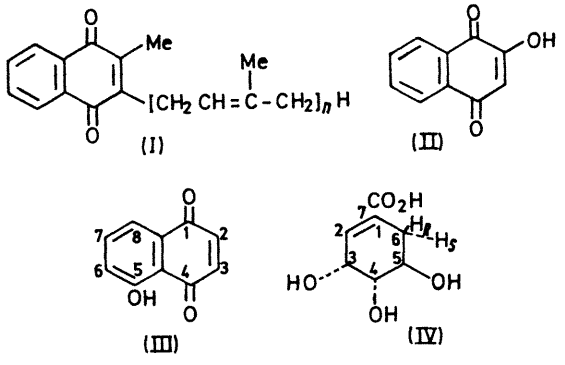
By K.-H. SCHARF and M. H. ZENK and (in part) D. K. ONDERKA, M. CARROLL, and H. G. FLOSS*

(*Institut für Pflanzenphysiologie der Ruhr-Universität Bochum, Germany*)

(*Department of Medicinal Chemistry and Pharmacognosy, Purdue University, Lafayette, Indiana* 47907)

Summary Feeding experiments with specifically and stereospecifically ^{14}C- and ^{3}H-labelled shikimic acids confirm earlier results on vitamin K and juglone formation and show that the biosynthesis involves loss of the *pro*-6*R* and retention of the *pro*-6*S* hydrogen of shikimate.

RECENT studies on the formation of vitamin K (I) and the biosynthetically related naphthoquinones lawsone (II) and juglone (III) have shown that the benzenoid ring of these compounds is derived from shikimic acid (IV).[1-6] It follows from the degradation of material obtained after [*U*-^{14}C]shikimate feeding that this precursor is incorporated with all its 7 carbon atoms, the carboxy-group providing one of the carbonyl carbon atoms of the quinones.[2-6] In



the case of juglone it was possible to demonstrate that the biosynthesis proceeds through a symmetrical intermediate in which the two carbonyl carbon atoms are indistinguishable.[5] The origin of the remaining three carbon atoms of the quinone ring was traced to carbon atoms 2, 3, and 4 of glutamate[7,8] and the site of their attachment was found to be C-2 of shikimate[9] in the case of juglone[5] and later also with the other two quinones.[10] [^{14}C]-*o*-Succinylbenzoic acid was efficiently incorporated into all three naphthaquinones,[11] suggesting its involvement in the biosynthesis, and some work with bacterial mutants lead to the suggestion[1,10,11] that chorismic acid might be an intermediate in the pathway. However, in a direct feeding experiment [^{14}C]chorismate was not incorporated into juglone while at the same time phenylalanine and tyrosine were efficiently labelled.[5]

We report results which further support some of these conclusions and present new evidence regarding the stereochemistry of naphthoquinone biosynthesis. Vitamin K_2 biosynthesis was studied with *Bacillus megaterium* mutant 248, a shikimic acid auxotroph,† and juglone biosynthesis in 6-week-old *Juglans regia* plants, using our previously described methods.[3,5] [6-^{14}C]- and [7-^{14}C]-shikimic acid, obtained enzymatically from [3-^{14}C]- and [1-^{14}C]-phosphoenolpyruvate,[12] gave vitamin K_2 (22·5 and 35·8% incorporation, respectively) which was degraded to phthalic acid. The latter upon Schmidt degradation gave anthranilic acid (92·3 and 53·3% of the radioactivity, respectively) and CO_2 (0 and 51·2% of the radioactivity). The anthranilate from the [6-^{14}C]shikimic acid experiment was further degraded to salicylic acid, 5-bromosalicylic acid, and 5-bromo-3-nitrosalicylic acid followed by the bromopicrin reaction to give bromopicrin from C-3 containing 46% of the radioactivity of the anthranilate. These experiments confirm that the shikimic acid carboxy-group is specifically incorporated into the carbonyl carbon atoms of vitamin K_2 and that the quinone ring is attached at C-2 of shikimic acid. The latter also follows from experiments with [2-^{3}H]shikimic acid prepared enzymatically from [4-^{3}H]erythrose-4-phosphate.[12] As shown in the first column of the Table, [7-^{14}C,2-^{3}H]shikimic acid is incorporated into vitamin K_2 and juglone with almost complete loss of the tritium, indicating that the hydrogen at C-2 is eliminated during the biosynthesis. Further experiments were done with shikimic acids carrying a stereospecific tritium label at C-6, which were obtained enzymatically from (*E*)- and (*Z*)-[3-^{3}H]phosphoenolpyruvate.[13] Each of these samples contained *ca.* 80—90% of its tritium in the

† We thank Dr. G. G. Gross of the laboratory at Bochum for the preparation of this mutant.

position indicated and the remainder in the diastereotopic position. As shown in the Table, most of the tritium from (6*S*)-[7-^{14}C,6-^{3}H] shikimate is retained during the biosynthesis of vitamin K_2, whereas the 6*R* specimen is incorporated with predominant loss of the tritium, indicating that the *pro*-6*R* hydrogen of shikimate is eliminated in above result does not involve an NIH shift, then leads to loss of half of the tritium. On the basis of these experiments, showing the elimination of the *pro*-6*R* and retention of the *pro*-6*S* hydrogen of shikimate, chorismic acid cannot be excluded as an intermediate in the biosynthesis of vitamin K_2 and juglone, because the formation of chorismic

TABLE. *Incorporation of doubly labelled shikimic acids into vitamin K_2 and juglone.*

Precursor:	[7-^{14}C,2-^{3}H]	(6*S*)-[7-^{14}C,6-^{3}H] Shikimic acid	(6*R*)-[7-^{14}C,6-^{3}H]
^{3}H/^{14}C of precursor	9·77	8·70	6·92
^{14}C incorporation (%)	17·1	15·05	15·0
^{3}H/^{14}C of vitamin K_2	0·62	7·30	1·29
Tritium retention (%)	6·3	84·0	18·6
^{3}H/^{14}C of precursor	11·95	5·77	5·27
^{14}C incorporation (%)	n.d.	0·52	0·27
^{3}H/^{14}C of juglone	0·60	2·75	0·08
Tritium retention (%)	5·0	47·6	1·5

n.d.: not determined.

naphthoquinone biosynthesis. The same is observed in juglone biosynthesis, but while the loss of tritium from the *pro*-6*R* position is almost complete, only about half of the tritium from the *pro*-6*S* position is incorporated in this case. This is additional evidence supporting the involvement of a symmetrical intermediate,[5,10] which results in an equal distribution of the tritium from the *pro*-6*S* position of shikimate between C-5 and C-8 of an unhydroxylated juglone precursor. Subsequent hydroxylation to 5-hydroxynaphthoquinone, which according to Leduc *et al.*[10] and the acid is known[13,14] to involve also elimination of the *pro*-6*R* and retention of the *pro*-6*S* hydrogen of shikimate.

Work at the Ruhr-University was supported by a grant from the "Ministerium für Bildung und Wissenschaft" and work at Purdue University by a research grant and a research career development award (to H. G. F.) from the National Institutes of Health.

(*Received, March 29th,* 1971; *Com.* 416.)

[1] G. B. Cox and F. Gibson, *Biochem. J.*, 1966, **100**, 1.
[2] M. H. Zenk and E. Leistner, *Z. Naturforsch.*, 1967, **22b**, 460.
[3] E. Leistner, J. H. Schmitt, and M. H. Zenk, *Biochem. Biophys. Res. Comm.*, 1967, **28**, 845.
[4] I. M. Campbell, C. J. Coscia, M. Kelsey, and R. Bentley, *Biochem. Biophys. Res. Comm.*, 1967, **28**, 25.
[5] E. Leistner and M. H. Zenk, *Z. Naturforsch.*, 1968, **23b**, 259.
[6] M. Guérin, M. M. Leduc, and R. G. Azerad, *European J. Biochem.*, 1970, **15**. 421.
[7] I. M. Campbell, *Tetrahedron Letters*, 1969, 4777.
[8] D. J. Robins, I. M. Campbell, and R. Bentley, *Biochem. Biophys. Res. Comm.*, 1970, **39**, 1081.
[9] The systematic numbering of shikimic acid (*J. Biol. Chem.*, 1968, **243**, 5809) is used rather than the traditional one.
[10] M. M. Leduc, P. M. Dansette and R. G. Azerad, *European J. Biochem.*, 1970, **15**, 428.
[11] P. Dansette and R. Azerad, *Biochem. Biophys. Res. Comm.*, 1970, **40**, 1090.
[12] K. H. Scharf and M. H. Zenk, manuscript in preparation.
[13] D. K. Onderka and H. G. Floss, *J. Amer. Chem. Soc.*, 1969, **91**, 5894.
[14] R. K. Hill and G. R. Newkome, *J. Amer. Chem. Soc.*, 1969, **91**, 5893.