A Revised Scheme for the Biosynthesis of Gossypol

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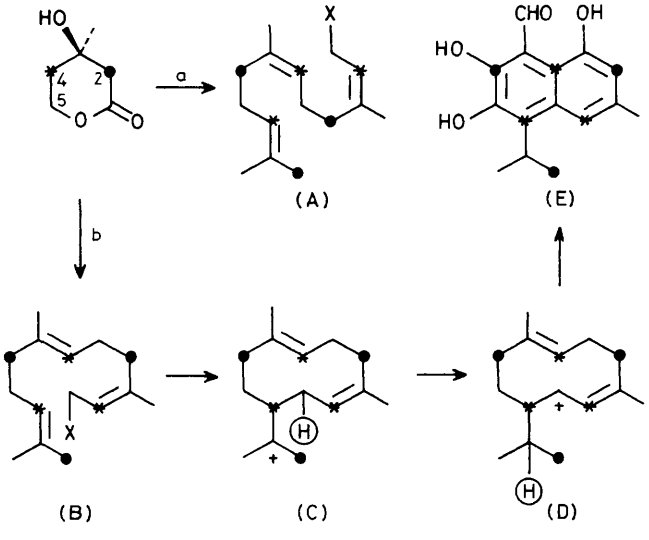
Chemical degradation of gossypol samples biosynthesized in cotton seedling roots from three differently labelled mevalonate precursors has revealed that, in contrast to previous claims, formation of this compound occurs as with other sesquiterpenes of the cadalane type *via* ten-membered ring cations correlated by a 1,3-hydride shift.

Gossypol (**1**), a pharmacologically interesting[1] bis-sesquiterpene from many *Gossypium* species, is formed by oxidative dimerization of hemigossypol[2] [(E), Scheme 1]. Extended studies on the biosynthesis of (**1**) by Heinstein *et al.*[3—5] have resulted in the claim that the intermediate (E) is formed preferentially from *cis,cis*-farnesylpyrophosphate folded as in (A). Since this scheme differs considerably from the one which has been established for the biosynthesis of all the other cadalane type sesquiterpenes investigated up to date,[6] (*cf.* path b, Scheme 1) a reinvestigation of the problem was deemed opportune. We now present new results which require a modification of the original proposal.



Scheme 1

Seeds of *Gossypium herbaceum* were germinated during 7 days following the procedure of Smith.[7] The roots were excised and transferred to the incubation flasks containing aqueous solutions of the radioactive precursors. After 10 days of incubation the roots were harvested and gossypol was isolated and purified as dianilinogossypol (**2**)[3,4] after dilution with cold carrier material.

Labelled dianilinogossypol (**2**) from the feeding of [2-^{14}C]-mevalonate was converted into the bisacetal (**3**) (mixture of stereoisomers).[8] Treatment of (**3**) with nitric acid led to gossic acid (**4**),[9] further characterized as its methyl ester (**5**), and to crude gossypolone (**6**), from which additional amounts of gossic acid were obtained by subsequent oxidation with potassium permanganate.[9] A second sample of labelled (**2**) was converted into the known apo-gossypolhexamethyl ether (**7**),[10] which upon treatment with sulphuric acid gave the desapo-derivative (**8**).[10] Oxidation of (**7**) by the RuO_4 method[11] yielded isobutyric acid (**10**), characterized as the

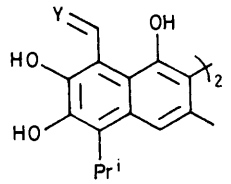
$$Me_2CHCO_2H$$
(10)

p-phenyl-phenacyl derivative. The observed radioactivity values (Table 1, expt. 1) confirm that the sesquiterpene intermediate is assembled from three mevalonate units; however, the loss of activity observed in going from (**3**) to (**4**) and (**5**) is in total contradiction with a previous result,[4] which

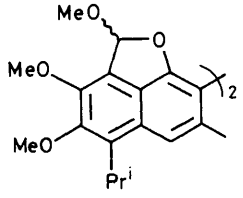
Table 1. Results of the degradation experiments with (**2**) from radioactively-labelled mevalonate (MVA) precursors.

Expt.	Precursor	% Incorporation	Relative molar activity (**3**)	(**7**)	(**8**)	(**4**)	(**5**)	(**10**)
1	[2-^{14}C]MVA[a]	0.51	6.00	6.24	4.15	3.92	3.98	1.01
2	[4-^{14}C]MVA[b]	1.80	6.00			6.06		

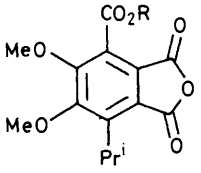
[a] 62 μg with total activity 24.7 μCi. [b] 50 mg with total activity 42.1 μCi.



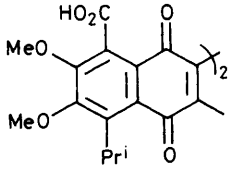
(**1**) Y = O
(**2**) Y = NC_6H_5



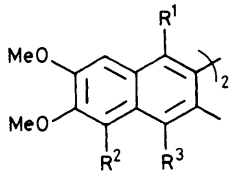
(**3**)



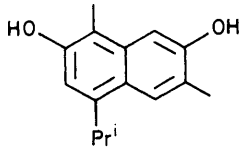
(**4**) R = H
(**5**) R = Me



(**6**)



(**7**) R^1 = OMe, R^2 = Pr^i, R^3 = H
(**8**) R^1 = OMe, R^2 = R^3 = H
(**9**) R^1 = R^3 = OCOMe, R^2 = Pr^i



(**11**)

was taken as a proof for the unusual cyclization path a (Scheme 1). Our measurements are compatible only with folding pattern (B) (Scheme 1) of the aliphatic precursor. Corroborative evidence for this cyclization mode was obtained by showing that gossypol biosynthesized from [4-^{14}C]mevalonate is degraded to gossic acid without loss of radioactivity (Table 1, expt. 2).

Finally, gossypol from a feeding experiment in which a mixture of [5-^{3}H]mevalonate and [2-^{14}C]mevalonate had been used was degraded to (**7**), from which the desapo-derivative (**8**)[10] and the tetra-acetate (**9**)[12] were obtained by known procedures. The radioactivity values listed in Table 2 indicate that in the course of hemigossypol biosynthesis one tritium equivalent from C-5 of the mevalonate precursor is preserved in the isopropyl side chain. This, together with the results mentioned above, is consistent with the operation of a 1,3-hydride shift [*cf.* (C) → (D), Scheme 1]. We therefore conclude that biosynthesis of gossypol matches in this detail

Table 2. Results of the degradation experiments with (**1**) from a mixture of radioactively-labelled mevalonate (MVA) precursors.

	Relative atomic ratio ^{3}H : ^{14}C
[2-^{14}C]MVA +[5-^{3}H]MVA[a]	2.00 : 1.00
(**7**)	2.07 : 6.00
(**8**)	0.00 : 4.00
(**9**)	1.99 : 6.00

[a] *Ca.* 2 mg with ^{14}C 24.7 μCi and ^{3}H 55.3 μCi.

the pathway already established for other sesquiterpenes of the cadalane type.

A folding pattern like the one now ascertained for the biosynthesis of (**1**) has been detected recently by Essenberg *et al.*[13] for the formation of (**11**), a closely related phytoalexin from bacterially infected cotton seedlings.

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