Fungal Metabolites. Part I. Stereochemical Features and Mass Spectrometry of Secalonic Acids
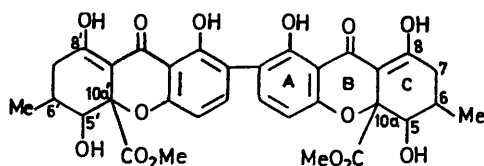
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The mass spectra of secalonic acids A, C, D, E(B) (dimethyl 5,5',6,6',7,7',10a,10a'-octahydro-1,1' 5 5',8,8'-hexahydroxy-6,6'-dimethyl-9,9'-dioxo-2,2'-bixanthen-10a,10a'-dicarboxylates) show features closely related to the stereochemistry at the six chiral centres in these compounds. Analysis of the spectra suggests that the stereochemistry of any new secalonic acid could be defined largely by its mass spectrum.

THE secalonic acids possess the general structure (I) and differ in stereochemical detail at positions 5, 6, 10a, 5', 6', and 10a'. Recently, in mass spectrometric investigations of stereoisomers, pronounced differences have been observed between pairs of isomers.[1] Franck[2]

		5-OH	5'-OH	6-Me	6'-Me	10a-CO$_2$Me	10a'-CO$_2$Me
(I)	A:	α	β	β	α	α	β
	C:	α	α	β	α	α	β
	D:	β	α	α	β	β	α
	E:	β	α	β	α	α	β

has suggested that loss of a methoxycarbonyl group from the molecular ion can be related to the orientation of this group with respect to the adjacent hydroxy-group in secalonic acids A and B. To some extent we confirm this observation, with reservations, and extend it to secalonic acids C, D, and E, and show also that other features of the mass spectra of these compounds may be

used to deduce the relative orientations of all the groups at positions 5, 6, 10a, 5', 6', and 10a'.

Results and Discussion.—The mass spectra of the secalonic acids A, C, D, and E were obtained both

TABLE 1

Element listings for secalonic acids, A, C, D, and E

		Relative abundances (%) *			
m/e	Composition	A	C	D	E
638	$C_{32}H_{30}O_{14}$	33·0	18·0	28·0	78·0
620	$C_{32}H_{28}O_{13}$	1·6	1·0	1·8	8·0
579	$C_{30}H_{27}O_{12}$	100·0	100·0	100·0	100·0
561	$C_{30}H_{25}O_{11}$	6·0	7·5	12·5	6·0
519	$C_{28}H_{23}O_{10}$	3·0	2·5	5·0	2·0
501	$C_{28}H_{21}O_9$	10·0	7·0	18·0	6·0
455	$C_{23}H_{19}O_{10}$		3·0	2·0	
260	$C_{14}H_{12}O_5$	20·0	5·5	21·0	14·0
183	$C_9H_{11}O_4$		2·5	6·0	
151	$C_8H_7O_3$	14·0	12·0	25·0	12·5
149	$C_8H_5O_3$	17·0	3·0	26·0	5·0
123	$C_7H_7O_2$	12·0	8·5	12·0	8·0
99	$C_5H_7O_2$	13·0	6·0	4·5	31·0
95	C_6H_7O	20·0	6·0	14·0	

* Ions of relative abundance <1% and of m/e <90 have been omitted.

routinely and by use of a data acquisition system to deduce the elemental compositions of the ions. Table 1 lists the compositions of the more abundant ions.

[1] For recent examples, see R. Hodges, R. C. Cambie, and K. N. Joblin, *Org. Mass Spectrometry*, 1970, **3**, 1473; V. I. Zaretskii, V. L. Sadovskaya, N. S. Wulfson, V. F. Sizoy, and V. G. Merimson, *ibid.*, 1971, **5**, 1179; H. Obermann, M. Spiteller-Friedmann, and G. Spiteller, *Tetrahedron*, 1971, **27**, 1093.

[2] B. Franck, E. M. Gottschalk, U. Ohnsorge, and F. Hüper, *Chem. Ber.*, 1966, **99**, 3842.

In secalonic acid E, the methoxycarbonyl and hydroxy-groups at positions 5 and 10a (and 5′ and 10a′) are *trans* and the abundances of the ions M (molecular) and $M - CO_2CH_3$ are in the ratio of $1 : 1 \cdot 2$ in the routine mass spectrum. However, in secalonic acid A the methoxycarbonyl and hydroxy-groups are *cis*-oriented and the ratio of abundances of the M and $M - CO_2CH_3$ ions is $1 : 3$.[2] Secalonic acid D, which is enantiomeric with A and has a *cis*-orientation of the methoxycarbonyl and hydroxy-groups at positions 5 and 10a, gives a mass spectrum in which the ratio of abundances of the M and $M - CO_2CH_3$ ions is about $1 : 3$. In secalonic acid C, the methoxycarbonyl and hydroxy-groups are *cis* to each other in one half of the molecule (as in secalonic acids B and E) and *trans* in the other half; the ratio of abundances of the M and $M - CO_2CH_3$ ions is $1 : 5 \cdot 5$ and not $1 : 2$ (mid-way between A and B) as might have been expected.

Franck has suggested that steric crowding is responsible for the observed difference in the relative abundances of the M and $M - CO_2CH_3$ ions in secalonic acids A and B, the greater relative abundance of the $M - CO_2CH_3$ ions being associated with a *cis* relationship between methoxycarbonyl and hydroxy-groups. We report here other features of the mass spectra which can be interpreted similarly.

Discussion.—The major decomposition pathways marked by metastable ion transitions are shown in Scheme 1.
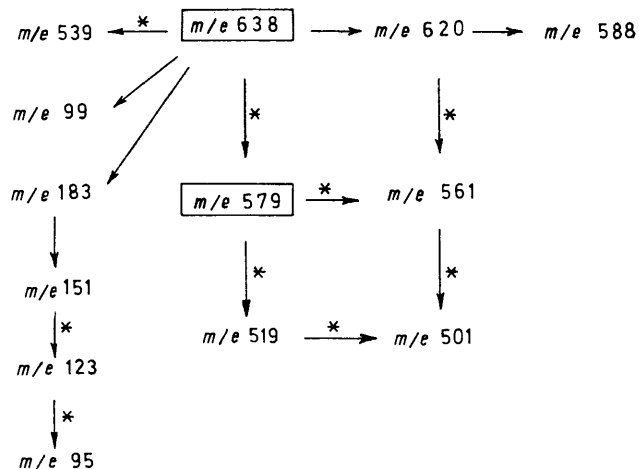
SCHEME 1　Metastable ion decompositions are indicated by an asterisk

When the mass spectra of secalonic acids A and E were measured on a second, different machine (MS 902 instead of MS 12) to obtain the element listings, the ratios of the M and $M - 59$ ion abundances changed markedly from those obtained on the first instrument (Table 2). Not only did the ratios change but also the secalonic acids A, D, and E were no longer distinguish-

able and, in each case, the abundance of the $M - 59$ ion was greater. This effect can be attributed to a greater ion source temperature (200°) in the MS 902 compared with the MS 12 instrument. When the mass spectrum of secalonic acid E was obtained on the MS 902 instrument at a lower temperature (180°), the ratio of abundances of M and $M - 59$ ions became closer to that obtained on the MS 12. Even at 16 eV (Table 2), the

TABLE 2

Ratios of relative abundances of M and $M - 59$ ions

Ratio M: $M - 59$	MS 12 (70 eV)	MS 902 (70 eV)	MS 902 (16 eV)	MS 12 (70 eV; ' corrected ')
A	$1 : 3 \cdot 0$	$1 : 4 \cdot 2$	$1 : 1 \cdot 5$	$1 : 1 \cdot 24$
C	$1 : 5 \cdot 5$	$1 : 6 \cdot 7$	$1 : 2 \cdot 6$	$1 : 2 \cdot 2$
D	$1 : 3 \cdot 6$	$1 : 4 \cdot 2$	$1 : 1 \cdot 4$	$1 : 1 \cdot 26$
E	$1 : 1 \cdot 3$	$1 : 4 \cdot 0$	$1 : 1 \cdot 1$	$1 : 0 \cdot 9$

abundance of the $M - 59$ was much greater than previously reported.[2] This effect of ion source temperature is well recognised in mass spectrometry[3] and emphasises the need for caution in defining the relative orientations of methoxycarbonyl and hydroxy-groups in the secalonic acids from the mass spectrum alone. In fact the ratio of abundances of the M and $M - 59$ ions is not necessarily easily interpretable. Naïvely, one might expect secalonic acid C, which can be regarded as half A and half B,[2] to have a ratio close to $1 : 2$. However the measured ratio is $1 : 5 \cdot 5$, which indicates the importance of other effects on the abundances of the M and $M - 59$ ions. Examination of the fragmentation pattern for the secalonic acids (Scheme 1) led us to sum the abundances of the ions at m/e 638, 620, 588, 539, 455, 183, 151, 149, 123, 99, and 95 to give an estimated abundance of the molecular ion as if it had not decomposed into these fragment ions. Similarly, summation of the ion abundances at m/e 579, 561, 519, 501, and 260 gave a ' corrected ' abundance for the ion at m/e 579. The ratios of these ' corrected ' abundances of the M (m/e 638) and $M - 59$ (m/e 579) ions for the secalonic acids A, C, D, and E are shown in Table 2. These ratios are remarkably close to those found at 16 eV when all competing fragmentations have been eliminated. Secalonic acids A and D are enantiomeric and give almost identical values, whereas E differs, and C gives a ratio very different from the other three. Thus there must be an extra energy term in secalonic acid C which does not appear in secalonic acids A and B, and this extra term is probably due to intramolecular interactions between the two halves of the molecule.

The molecular ion eliminated H_2O to a much greater extent in secalonic acid E than in A, D, or C. The loss of H_2O in mass spectrometry is known[4] to require the presence of a hydrogen atom on a γ- or δ-carbon atom, and these occur at the methyl in position 6 and at C-7.

[3] For examples see, G. Spiteller and M. Spiteller-Friedmann in ' Some Newer Physical Methods in Structural Chemistry,' eds. R. Bonnet and J. G. Davies, United Trade Press, Lindon, 1967, p. 121; S. Meyerson and A. W. Weitkamp, *Org. Mass Spectrometry*, 1968, **1**, 659.

[4] W. H. McFadden, M. Lounsbury, and A. L. Wahrhaftig, *Canad. J. Chem.*, 1958, **36**, 990; W. H. McFadden, D. R. Black, and J. W. Corse, *J. Phys. Chem.*, 1963, **67**, 1517; C. G. MacDonald, J. S. Shannon, and G. Sugowdz, *Tetrahedron Letters*, 1963, 807; W. Benz and K. Biemann, *J. Amer. Chem. Soc.*, 1964, **86**, 2375; S. Meyerson and L. C. Leitch, *ibid.*, p. 2555.

It is also known that secondary hydrogen atoms take part in the elimination more readily than primary hydrogens,[5] and therefore we can suppose that the H_2O eliminated from the molecular ion originates from the OH at C-5 and a hydrogen atom at C-7. The more abundant elimination of H_2O in secalonic acid E compared with secalonic acid A is then readily ascribable to

SCHEME 2
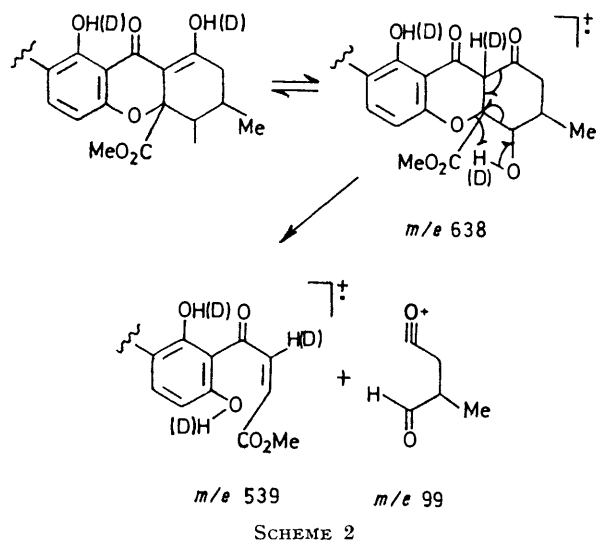
the closer proximity of the hydroxy-group to the C-7 protons in E. The mass spectra of the secalonic acids appear therefore also to yield information on the orientation of the hydroxy-group at C-5. Again, secalonic acid C does not behave as half A and half B: the relative abundance of the $M - H_2O$ ion is less than in either A or B. If the configuration of the centre at C-6 has been determined by oxidation experiments [6] as R or S, then the mass spectrum can provide a good indication of the remainder of the stereochemistry at C-5(5') and C-6(6') in secalonic acids.

It is possible that the $M - 18$ ion observed in these spectra arises, at least partly, by thermal elimination of water from the sample during vaporisation into the ion source (see also later). It is fortunate that the same orientation of hydroxy and methyl (cis) favouring loss of H_2O as a mass spectrometric process is also that favourable for the acid-catalysed trans-elimination of H_2O in a ground-state process. The point underlines the need to examine the mass spectrum of any new secalonic acid under carefully controlled ion-source conditions and preferably with direct reference to the mass spectrum of any available isomer run under identical conditions.

Further evidence for the effect of stereochemistry on the mass spectra of the secalonic acids can be found in the abundance of the ion at m/e 99. This ion appears to arise directly from the molecular ion by the fragmentation shown in Scheme 2. A mechanism such as this is

supported by the elemental composition of the ion at m/e 99, which indicates its origin, and the existence of a small abundance of an ion at m/e 539 (539 + 99 = 638, the molecular weight). On exchanging the hydroxy-protons for deuterium, no shift was observed in the ion at m/e 99, thus demonstrating the origins of the transferred hydrogen atoms. In the case of the 8,8'-di-O-methyl ethers of the secalonic acids, the ion at m/e 99 is not formed, nor is the corresponding ion shifted by 14 mass units observed; this shows the importance of the hydrogen transfer process in its formation. The abundance of the ion at m/e 99 is much greater in secalonic acid E than in A and D. The formation of the ion at m/e 99 from the molecular ion competes with the ready formation of the ion at m/e 579 from the molecular ion. The abundance of the ion at m/e 99 can be expected to be greater in secalonic acid A because the major competing fragmentation, loss of 59 mass units, is a weaker process. The abundance of the ion at m/e 99 appears atypical in secalonic acid C.

Franck has reported [2] a series of abundant ions at m/e 166, 135, and 107 in the spectra of secalonic acids A and B (Scheme 3). Although we observe these ions in the mass spectra of secalonic acids A, C, D, and E their abundances are insignificant. Instead of this series of ions we observe the series shown in Scheme 4 which Franck does not report. Comparison of Schemes 3 and 4 shows that the origin of these two sets of ions is considered to be the same, the difference being the presence or absence of a hydroxy-group. The difference in the two sets of results is probably due, in Franck's
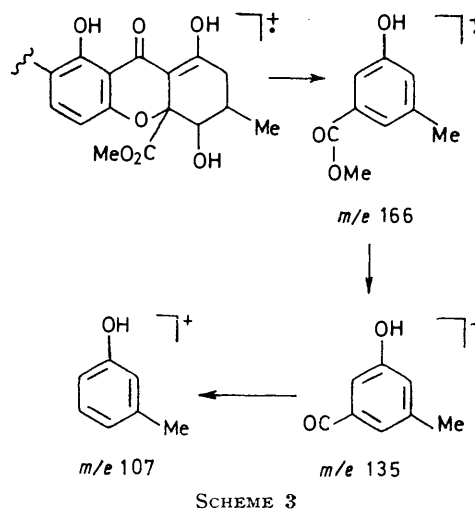
SCHEME 3

work, to thermolytic elimination of OH from position 5 as H_2O during vaporisation of the sample into the ion chamber. The origin of the ions m/e 183 ⟶ 95 (Scheme 4) is evident from their elemental compositions,

[5] H. Budzikiewicz, C. Fenselau, and C. Djerassi, Tetrahedron, 1966, 22, 1391.

[6] See ref. 2 and D. J. Aberhart, Y. S. Chan, P. de Mayo, and J. B. Stothers, Tetrahedron, 1965, 21, 1417; P. S. Steyn, ibid., 1970, 26, 51; J. W. ApSimon, J. A. Corran, N. G. Creasy, W. Marlow, W. B. Whalley, and K. Y. Sim, J. Chem. Soc., 1965, 4144.

metastable ion transitions, and behaviour on deuteri-ation. In hexadeuteriated secalonic acid A, the ions at m/e 183 $\longrightarrow$ 95 increase partly by one mass unit, consistent with the Scheme shown.
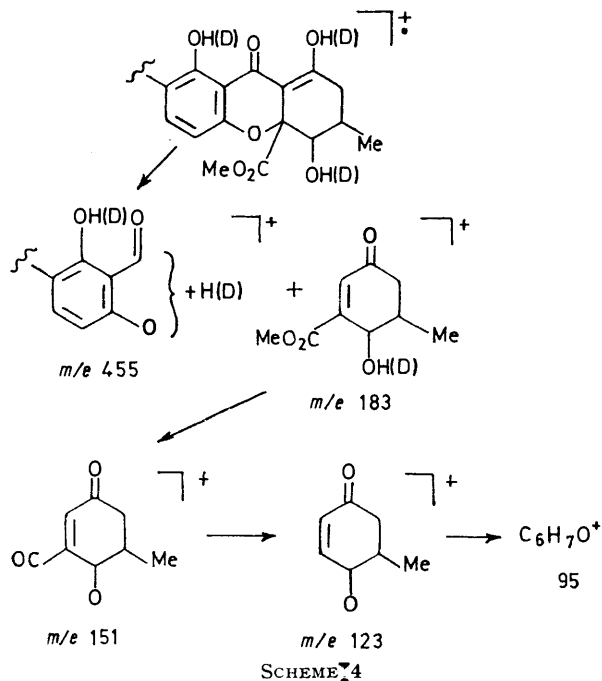
SCHEME 4

The other major ions at m/e 561, 519, and 501 in the mass spectra of the secalonic acids arise as shown in Scheme 1, but do not seem to show any marked stereo-chemical effects. This is in keeping with their formation from the ion at m/e 579, since formation of the latter uses up steric compression energy. The ion at m/e 260

is due to loss of CO_2CH_3 from the doubly-charged molecular ion.

Conclusion.—Structural features of the secalonic acids are reflected in their mass spectra. Ring c (I) is ejected as a whole from the molecular ion and the amount of loss is influenced by the relative orientations of the methoxycarbonyl and hydroxy-groups at C-10a and C-5. The orientation of these groups also affects the abund-ance of the $M - 59$ ion but care to keep the ion source temperature down to 180° or less is necessary to obtain meaningful results. The $M - 18$ ion can be used to determine the relative orientations of the hydroxy- and methyl groups at C-5 and C-6. When the secalonic acid is not enantiomeric about the biphenyl linkage, these stereochemical effects appear to be compound, rather than simple averages.

EXPERIMENTAL