corresponding β substituents, while in mobile systems the conformational preference of the chromophore may be indicated.

Experimental Section

The nuclear magnetic resonance spectra were determined using a Varian HA-100 spectrometer equipped with variable-temperature probe. The variable-temperature system was calibrated by measuring peak separations in the spectrum of ethylene glycol (for the range +60 to +100°) or in the spectrum of methanol (for the range −80 to +60°); the specified calibration accuracy is ±3°. Peak positions were measured directly from calibrated paper, after checking the calibration using a frequency counter (Advance, timer counter Type TC2A) immediately before each run was performed. Resonance positions were measured relative to an internal tetramethylsilane reference. The spectra of all the compounds were determined using dilute solutions (2–5% w/v) in carbon tetrachloride or in toluene-d_8; the latter solvent was obtained from Merck Sharp and Dohme, Montreal. Spectral analysis was carried out using the first-order method; analysis of the ABC system of but-3-en-2-one (XII) was aided by the spectrum of 1,1,1,3-d_4-but-3-en-2-one.

Acknowledgments. J. R. is grateful for the award of an SRC postgraduate studentship. We wish to thank Mr. M. Karger and Dr. I. Fleming for a sample of 1,1,1,-3-d_4-but-3-en-2-one. Thanks are expressed to Dr. A. F. Thomas (Firmenich et Cie, Geneva) for a generous gift of 3-methylcyclohexenone.

Structural Elucidation and High-Resolution Mass Spectrometry of Gaillardin, a New Cytotoxic Sesquiterpene Lactone

S. Morris Kupchan,[1] John M. Cassady,[1,2] John E. Kelsey,[1,4] H. K. Schnoes,[3] D. H. Smith,[3,5] and A. L. Burlingame[3]

Contribution from the Department of Pharmaceutical Chemistry, University of Wisconsin, Madison, Wisconsin 53706, and the Space Sciences Laboratory and Department of Chemistry, University of California, Berkeley, California.
Received June 15, 1966

Abstract: Evidence is presented for assignment of structure **6** to gaillardin. Elemental analysis and high-resolution mass spectrometry supported a $C_{17}H_{22}O_5$ molecular formula for gaillardin. Chemical and spectral evidence indicated the presence of α,β-unsaturated lactone, acetate ester, tertiary hydroxyl, and isolated double-bond groupings. The perhydroazulene ring system was indicated by dehydrogenation to chamazulene (**7**). Partial hydrogenation or sodium borohydride reduction of gaillardin (**6**) gave dihydrogaillardin (**9**), and epoxidation of **9** gave dihydroepoxygaillardin (**12**). Alkaline hydrolysis of **9** afforded desacetyldihydrogaillardin (**11**) and oxidation of **11** gave desacetyldihydrodehydrogaillardin (**14**). Dehydration of **14** gave dienones **13** and **15**. Treatment of gaillardin (**6**) with aqueous methanolic potassium carbonate gave 13-methoxydesacetylgaillardin (**5**); hydrolysis in aqueous dioxane gave desacetylgaillardin (**8**). Catalytic hydrogenation of gaillardin (**6**) gave tetrahydrogaillardin (**10**). Low- and high-resolution mass spectra of gaillardin (**6**) and a number of its derivatives (**5**, **8**, **9**, **11**, **12**, and **14**) are presented and discussed. The mass spectra of the desacetyl derivatives **8** and **11** are interpreted in detail.

Gaillardin is a cytotoxic sesquiterpene lactone from *Gaillardia pulchella* Foug., and its isolation and preliminary characterization have recently been reported.[6,7] It is the purpose of this paper to present in detail the structural elucidation and high-resolution mass spectrometry of gaillardin (**6**). Gaillardin appears to be the first normal guaianolide derivative isolated from *Gaillardia* species.

The molecular formula, $C_{17}H_{22}O_5$, was assigned for gaillardin on the basis of elemental analysis and high-resolution mass spectrometry. The presence of high intensity end absorption in the ultraviolet absorption spectrum and of bands at 5.67 and 6.00 μ in the infrared absorption spectrum suggested the presence of an α,β-unsaturated lactone group **1**. The latter grouping is also found in other constituents of *Gaillardia* species.[8–10] The presence of an acetate grouping, indicated by the molecular formula and the infrared absorption at 5.78 and 8.15 μ, was supported by the presence of a characteristic M − 60 peak at *m/e* 246 in the mass spectrum of gaillardin. The infrared (2.78 μ) and mass (M − 18 peak) spectra also indicated the presence of a hydroxyl group. The resistance of the hydroxyl group toward attempted acetylation with acetic anhydride–

(1) University of Wisconsin. This is part XVII in the series entitled "Tumor Inhibitors." Part XVI is: S. M. Kupchan, Y. Aynehchi, J. M. Cassady, A. T. McPhail, G. A. Sim, H. K. Schnoes, and A. L. Burlingame, *J. Am. Chem. Soc.*, **88**, 3674 (1966). The investigation at the University of Wisconsin was supported in part by grants from the National Institutes of Health (CA-04500) and the American Cancer Society (T-275).

(2) National Institutes of Health Postdoctoral Fellow, 1965–1966.

(3) University of California. This is part VIII in the series entitled "High Resolution Mass Spectrometry in Molecular Structure Studies." Part VII: A. L. Burlingame, R. W. Olsen, K. L. Pering, H. M. Fales, and R. J. Highet, in preparation. The investigation at the University of California was supported in part by a grant from the National Aeronautics and Space Administration (NsG 101).

(4) National Institutes of Health Predoctoral Fellow, 1965–1966.

(5) National Aeronautics and Space Administration Predoctoral Fellow, 1965–1966.

(6) S. M. Kupchan, J. M. Cassady, J. Bailey, and J. R. Knox, *J. Pharm. Sci.*, 54, 1703 (1965).

(7) Roots, stems, leaves, and flowers were collected 8–9 miles southwest of George West along Route 59, Live Oak County, Texas, on April 13, 1964. The authors acknowledge with thanks receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture, Beltsville, Md., in accordance with the program developed with the U. S. D. A. by the Cancer Chemotherapy National Service Center.

(8) W. Herz, K. Ueda, and S. Inayama, *Tetrahedron*, **19**, 483 (1963).

(9) W. Herz and S. Inayama, *ibid.*, **20**, 341 (1964).

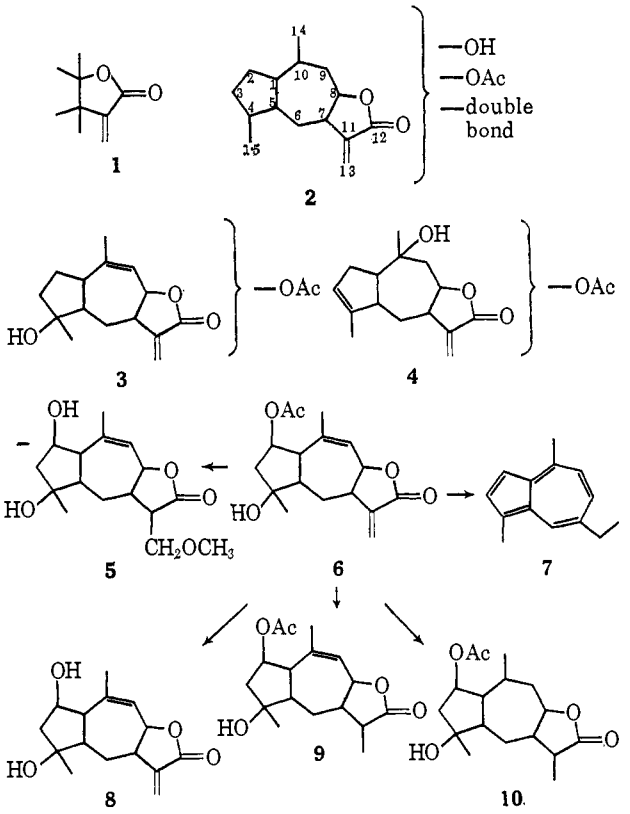
(10) W. Herz, S. Rajappa, M. V. Lakshmikantham, and J. J. Schmid, *ibid.*, **22**, 693 (1966).

Table I. Nmr Signals[a]

Compound	C-2 H	C-3 H	C-4 CH_3	C-8 H	C-9 H	C-10 CH_3	C-13 H	C-2 OAc
6	4.70 q[b]		8.74 s	5.50 m[c]	4.07 brs	8.18 brs	3.79 d (3) 4.47 d (3)	7.92 s
9	4.67 q[b]		8.77 s	5.50 m[c]	4.12 brs	8.18 brs	8.78 d (7)[d]	7.90 s
11	5.63 m[e]		8.73 s	5.43 m[e]	4.18 brs	7.94 brs	8.77 d (6)[d]	
14[f]		7.43 s	8.74 s	5.32 m[c]	4.21 brs	8.23 brs	8.77 d (6.5)[d]	
15[g]		3.91 brs	7.56 s	6.16 m[c]	7.27 d (6)[d]	7.89 s	8.71 d (7)[d]	
10	4.83 m		8.80 s	5.92 brm		8.86 d (7)	8.78 (6)[d]	7.90 s
8	5.60 m		8.74 s	5.50 m	4.14 brs	7.90 brs	3.79 d (3) 4.47 d (3)	
5[h]	5.65 m		8.75 s	5.50 m	4.16 brs	7.92 brs		
12	4.72 q[b]		8.83 s	5.77 d (9)	6.77 s	8.63 s	8.75 d (6)[d]	7.88 s

[a] Spectra determined on a Varian A-60 spectrometer in deuteriochloroform. Values are given in τ units relative to tetramethylsilane as internal standard. Numbers in parentheses denote coupling constants in cps. Multiplicity of signals is designated as follows: s, singlet; d, doublet; q, quartet; m, multiplet center; brs, broad singlet; brm, broad multiplet. [b] Deformed. [c] Diffuse doublet center. [d] Center. [e] C-2 H and C-8 H overlap. [f] C-1 H 7.11 d (11). [g] C-5 H 6.89 doublet of doublets. [h] Protons in OCH_3, 6.61 s; $-CH_2-O-$, 6.30 d.

pyridine supported assignment to a tertiary position. The presence of a second double bond was revealed by a weak absorption at 6.04 μ in the infrared spectrum, and the presence of two double bonds was confirmed

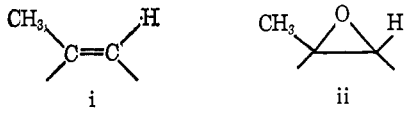


1 2 3 4 5 6 7 8 9 10

by the fact that 2 mole equiv of hydrogen was absorbed upon catalytic hydrogenation of gaillardin. Inspection of the molecular formula in the light of the functional groups known to be present indicated that gaillardin is tricyclic.

The 5,7 or perhydroazulene ring system was favored by the fact that all *Gaillardia* constituents characterized earlier have been of this type. The nature of the carbon skeleton was confirmed by dehydrogenation of gaillardin, which yielded chamazulene (**7**)[11] in relatively good yield. This made possible adoption of partial formula **2** as a working hypothesis, with ring closure to C-8 indicated by the multiplicity of the lactone proton signal in the nmr spectrum of gaillardin.

The nmr spectra (see Table I) of gaillardin and its derivatives were very instructive. The spectrum of gaillardin (**6**) showed the typical low-field doublets of the exocyclic methylene group at τ 3.79 and 4.47 (J = 3 cps). The latter signals were absent from the spectrum of dihydrogaillardin (**9**), which showed a methyl doublet at τ 8.78 (J = 7 cps). A diffuse singlet at τ 4.07 which remained in the spectrum of the dihydro derivative (but disappeared in that of tetrahydrogaillardin (**10**)) was assigned to a vinyl proton. A broad methyl singlet at τ 8.18 indicated that the double bond was also substituted with a methyl group (i). This partial structure was confirmed by epoxi-



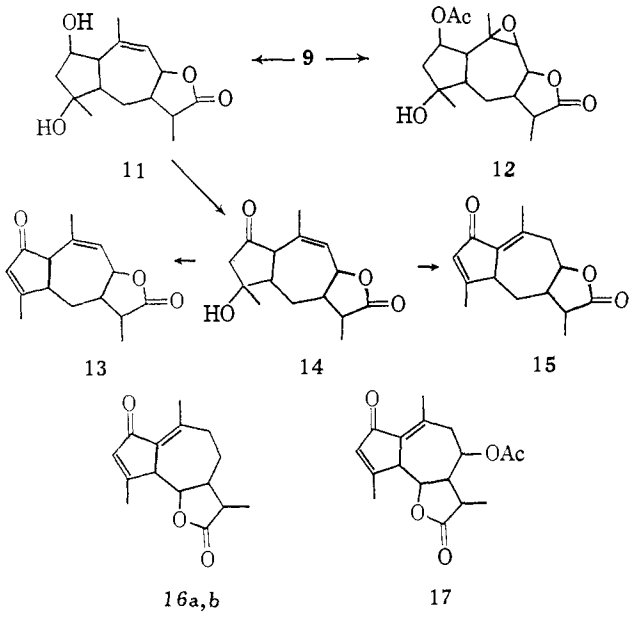
i ii

dation of dihydrogaillardin to give **12**, in the spectrum of which the C-14 and C-9 proton signals appeared at higher field. The methyl group appeared as a sharp singlet at τ 8.63 while a singlet at τ 6.77 indicated the presence of a proton on carbon attached to the epoxide ii. Two additional low-field protons appeared in the nmr spectrum of gaillardin (**6**), a multiplet at τ 4.70 and a diffuse doublet (J = 10 cps) at τ 5.50. The signal at lower field was tentatively assigned to the proton on carbon bearing the acetate group. This assignment was confirmed by the nmr spectra of desacetylgaillardin (**8**), methoxydesacetylgaillardin (**5**), and desacetyldihydrogaillardin (**11**) in which the signal at τ 5.50 remained but the signal at τ 4.70 had been replaced by a signal upfield at *ca.* τ 5.60. The signal at τ 5.50 which remained invariant in **8**, **5**, and **11** thus could be assigned to the lactonic proton at C-8. The absence of further low-field protons confirmed the tertiary nature of the hydroxyl group and, in conjunction with the presence of a methyl singlet at τ 8.74,[12] indicated that the tertiary hydroxyl group is attached to a carbon atom bearing a methyl group (>C(—OH)—CH_3). A sharp singlet at τ 7.92 was characteristic of an acetate methyl group. The nmr spectra thus supported formula **2** and allowed expansion to **3** or **4**.

(11) A. Meisels and A. Weizmann, *J. Am. Chem. Soc.*, 75, 3865 (1953).

(12) V. Prochazka, Z. Cekan, and R. B. Bates, *Collection Czech. Chem. Commun.*, 28, 1204 (1963).

Further examination of the nmr spectra of tetrahydrogaillardin (**10**) and dihydroepoxygaillardin (**12**) supported assignment of partial formula **3**. The lactonic hydrogen in the tetrahydro derivative appeared as a complex multiplet centered at τ 5.92 in contrast to the diffuse doublet at *ca.* τ 5.50 in **6** and **9**, a shift typically observed for an allylic proton on reduction. The presence of the 9,10 double bond in gaillardin, adjacent to the lactonic proton at C-8, was further confirmed by the nmr spectrum of the epoxide **12**, in which the lactone proton signal appeared as a clean doublet at τ 5.77 (J = 9 cps). This coupling is clearly between the C-7



and C-8 protons, as the epoxide proton at C-9 appears as a clean singlet, indicating a dihedral angle between the protons on C-8 and C-9 of approximately 90°.

The structural problem which remained at this point was the choice between C-2, C-3, and C-6 as the position of attachment of the secondary acetate group. This was accomplished by the following sequence. Treatment of gaillardin (**6**) with sodium borohydride in methanol at room temperature[13] gave dihydrogaillardin, $C_{17}H_{24}O_5$ (**9**). This compound showed absorption at 2.88 (hydroxyl), 5.63 (lactone carbonyl), 5.76, 8.02 (acetate), and 6.03 μ (double bond) in the infrared, and no absorption characteristic of an α,β-unsaturated lactone in the ultraviolet. Hydrolysis gave desacetyldihydrogaillardin (**11**), $C_{15}H_{22}O_4$, which showed no absorption at 5.76 and 8.02 μ in the infrared and no acetate methyl signal in the nmr. Chromic acid oxidation of **11** gave a ketone (**14**), $C_{15}H_{20}O_4$, which showed absorption at 5.66 (lactone) and 5.75 μ in the infrared, indicating the presence of a cyclopentanone moiety. The ultraviolet spectrum showed end absorption (ϵ 6050) at 212 mμ, characteristic of a β,γ-unsaturated ketone.[14] These observations are consistent only with substitution of the acetate group at C-2 and thus support assignment of structure **14** for the ketone. The nmr spectrum was consistent with this structure since the signal for the proton at C-2 had disappeared, that for the C-3 methylene appeared as a singlet at τ 7.43, and the C-1 proton appeared as a doublet at τ 7.11 (J = 11 cps) (while the signals for the protons at C-8 and C-9 and the methyl groups remained invariant).

Treatment of the ketone **14** with thionyl chloride–pyridine gave homogeneous but noncrystalline α,β-unsaturated ketone **13** which showed absorption at 5.62 (lactone) and 5.90 μ (α,β-unsaturated ketone) in the infrared spectrum (*cf.* the spectrum of dihydroachillin[15]). Treatment of the ketone **14** with *p*-toluenesulfonic acid in benzene under reflux gave the cross-conjugated cyclopentadienone **15**, $C_{15}H_{18}O_3$. This compound showed absorption in the ultraviolet at 255 mμ (ϵ 16,600) and infrared absorption at 5.63 (s), 5.95 (s), 6.11 (m), and 6.18 (s) μ, typical of that found in achillin (**16a**),[15] leukodin (**16b**),[16] and matricarin (**17**).[17,18] The nmr spectrum of the dienone (**15**) fully supports the assigned structure (*cf.* the nmr spectrum of achillin[15]). The only significant difference in the nmr spectra of these compounds results from the point of attachment of the lactone ring (C-6 in achillin (**16a**), τ 6.16, triplet; C-8 in **15**, τ 6.12, complex multiplet). In addition, the signal for the C-5 proton in achillin (**16a**) appears as a doublet at τ 6.66 (J = 10 cps) while in **15** the signal for this proton is a doublet of doublets at τ 6.89. The arguments outlined above for assignment of partial formula **3**, in conjunction with the conversion to the dieneone **15** just described, lead to proposal of **6** as the structure of gaillardin.

A companion sesquiterpene lactone, isogaillardin, $C_{17}H_{22}O_5$, mp 140–141°, $[\alpha]^{27}D$ +113° (*c* 1.21, $CHCl_3$), has been isolated from the same fraction which gives gaillardin. The structural elucidation of isogaillardin, which also shows significant cytotoxic activity, is now in progress.

Mass Spectrometry

Sesquiterpenes have not been studied extensively by mass spectrometry and it seems appropriate therefore to discuss some of the more important features of the mass spectra of gaillardin and derivatives, particularly since the complete high-resolution spectra of these compounds permit some reasonable deductions concerning their ionic decomposition. Brief discussions of the mass spectra of santonin and derivatives[19,20] and some other sesquiterpene systems[20–22] have appeared, and a more extensive treatment of the sesquiterpene widdrol has been presented.[23]

In the course of this study, we have obtained both low- and high-resolution mass spectra of gaillardin (**6**), desacetylmethoxygaillardin (**5**), desacetylgaillardin (**8**), di-

(13) R. C. Lucas, S. Rovinski, R. J. Kiesel, L. Dorfman, and H. B. MacPhillamy, *J. Org. Chem.*, **29**, 1549 (1964).

(14) *Cf.* H. Labhart and G. Waginere, *Helv. Chim. Acta*, **42**, 2219 (1959).

(15) E. H. White and R. E. K. Winter, *Tetrahedron Letters*, 137 (1963).

(16) M. Holub and V. Herout, *Collection Czech. Chem. Commun.*, **27**, 2980 (1962).

(17) Z. Cekan, V. Prochazka, V. Herout, and F. Sorm, *ibid.*, **24**, 1554 (1959).

(18) W. Herz and K. Ueda, *J. Am. Chem. Soc.*, **83**, 1139 (1961).

(19) N. Wasada, T. Tsuchiya, E. Yoshii, and E. Watanabe, paper presented at the 13th Annual Conference on Mass Spectrometry and Allied Topics, St. Louis, Mo., May 16–21, 1965.

(20) D. G. B. Boocock and E. S. Waight, *Chem. Commun.*, 90 (1966).

(21) R. I. Reed in "Mass Spectrometry of Organic Ions," F. W. McLafferty, Ed., Academic Press Inc., New York, N. Y., 1963, Chapter 13.

(22) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day Inc., San Francisco, Calif., 1964, Chapter 23.

(23) C. F. Fenselau, W. Richter, and A. L. Burlingame, 14th Annual Conference on Mass Spectrometry and Allied Topics, Dallas, Texas, June 21–25, 1966 (full paper in preparation).

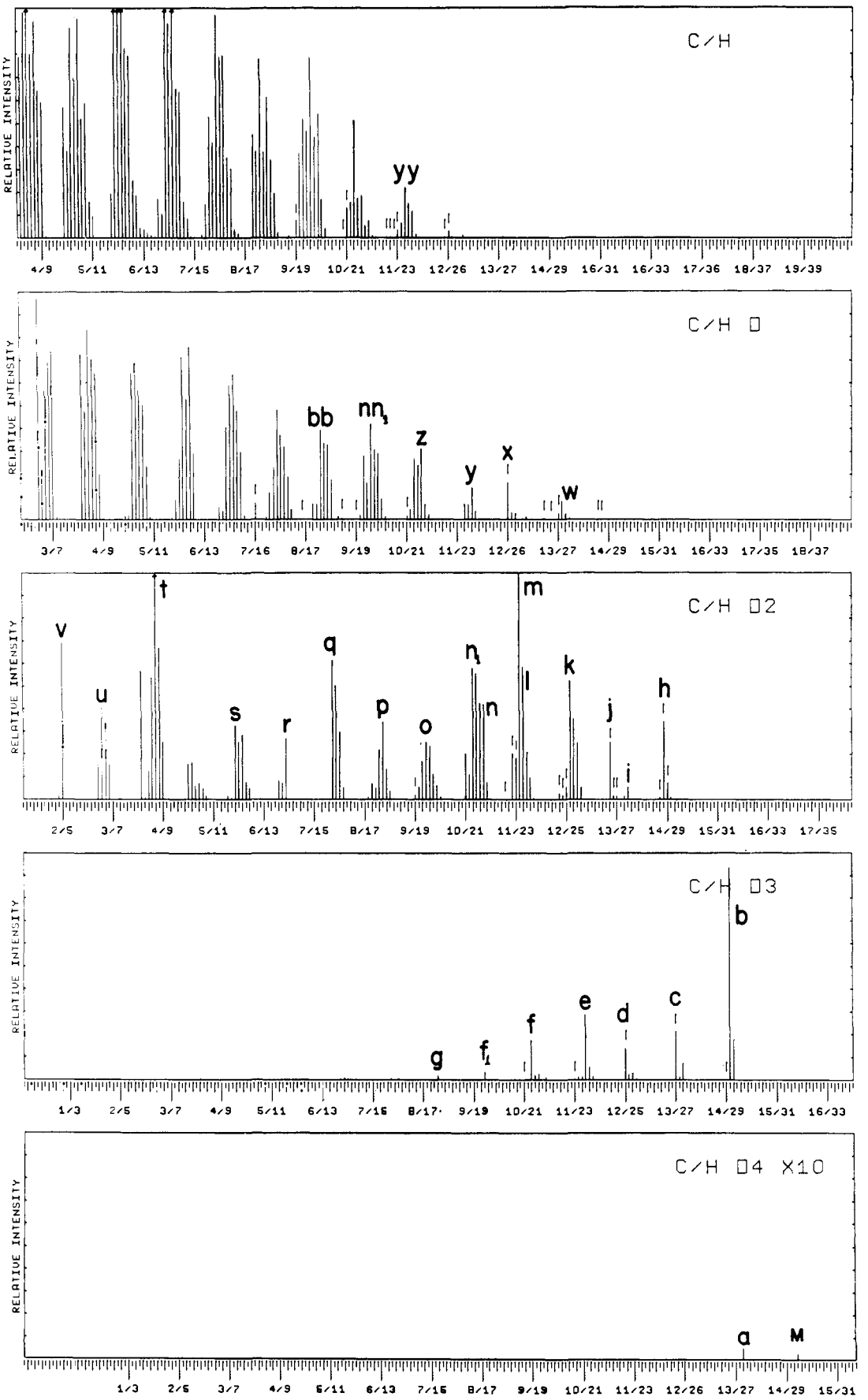


Figure 1. High-resolution mass spectrum of desacetylgaillardin (**8**).

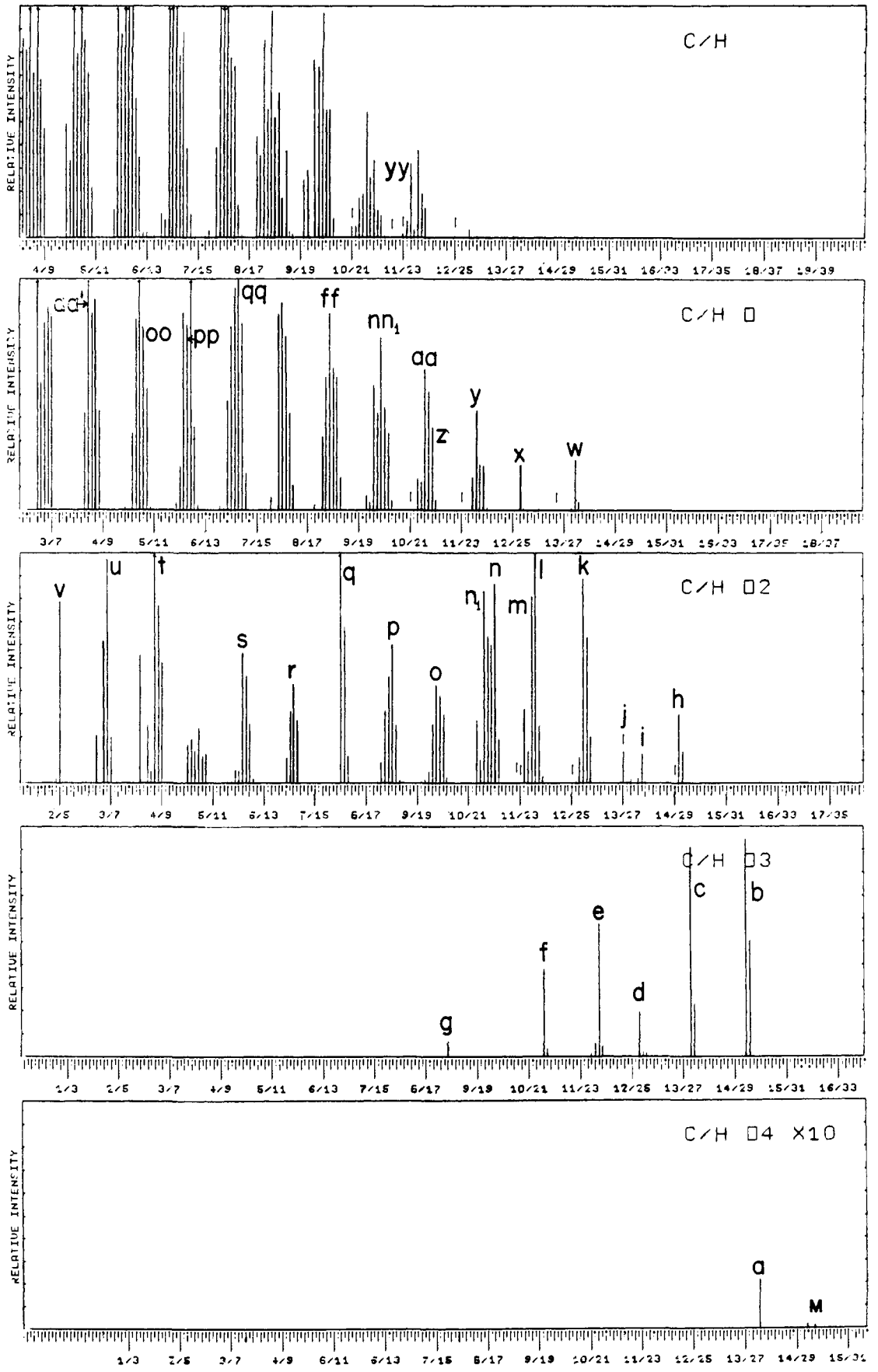


Figure 2. High-resolution mass spectrum of desacetyldihydrogaillardin (**11**).

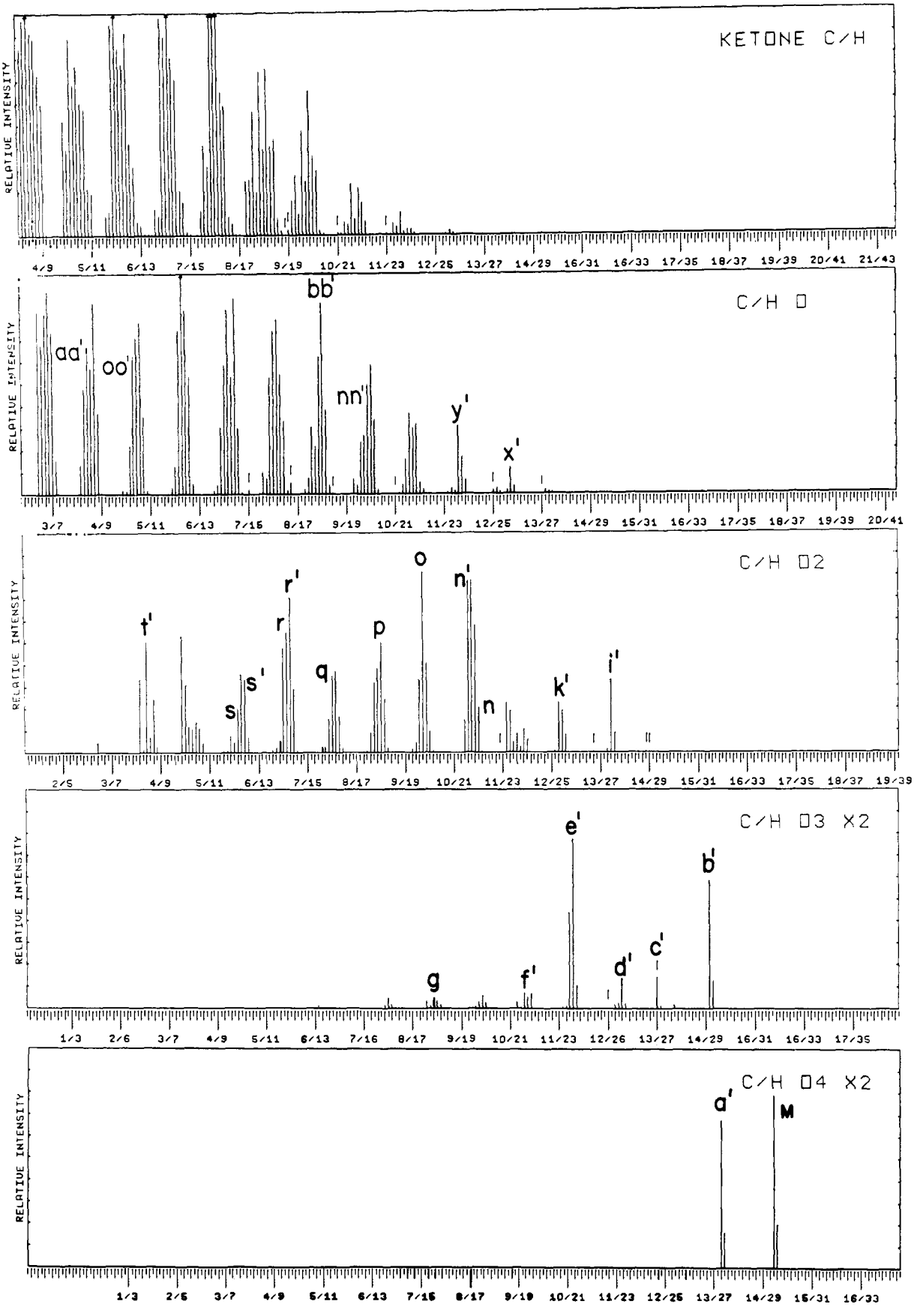


Figure 3. High-resolution mass spectrum of ketone **14**.

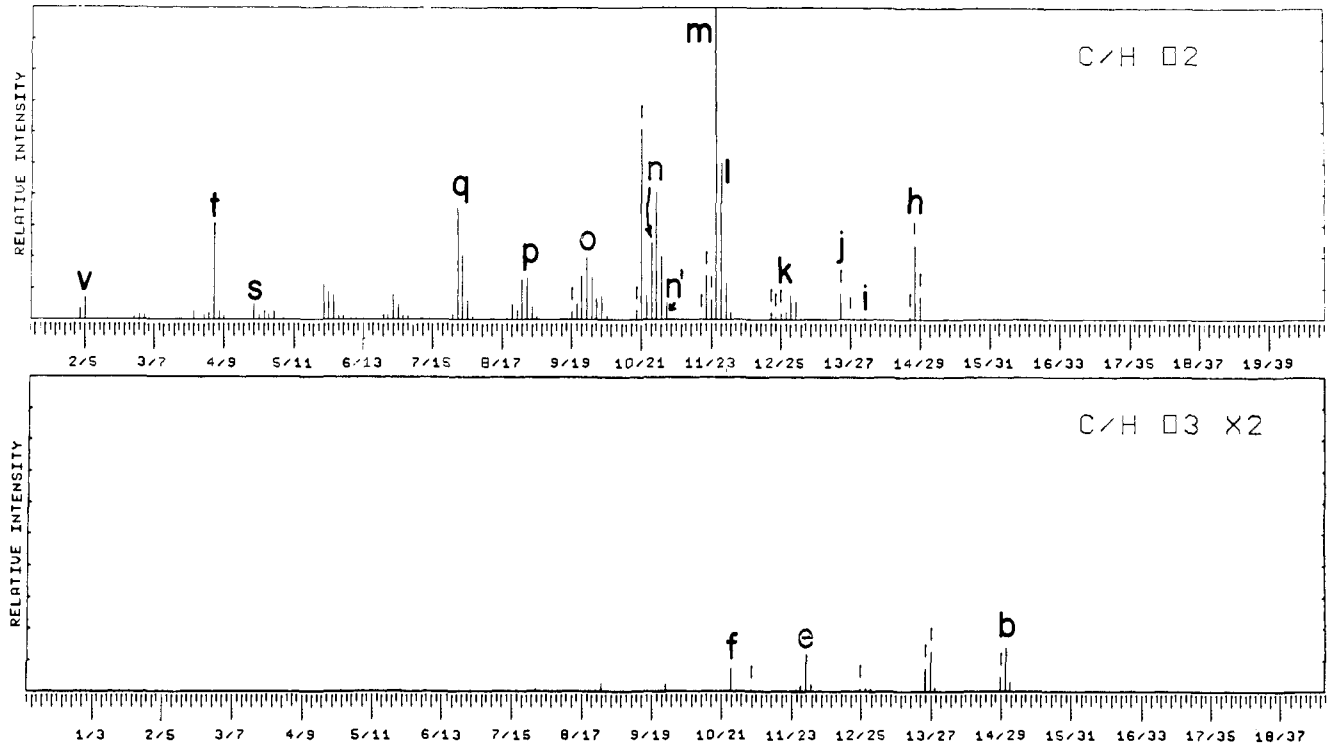


Figure 4. Partial high-resolution mass spectrum of gaillardin (**6**).

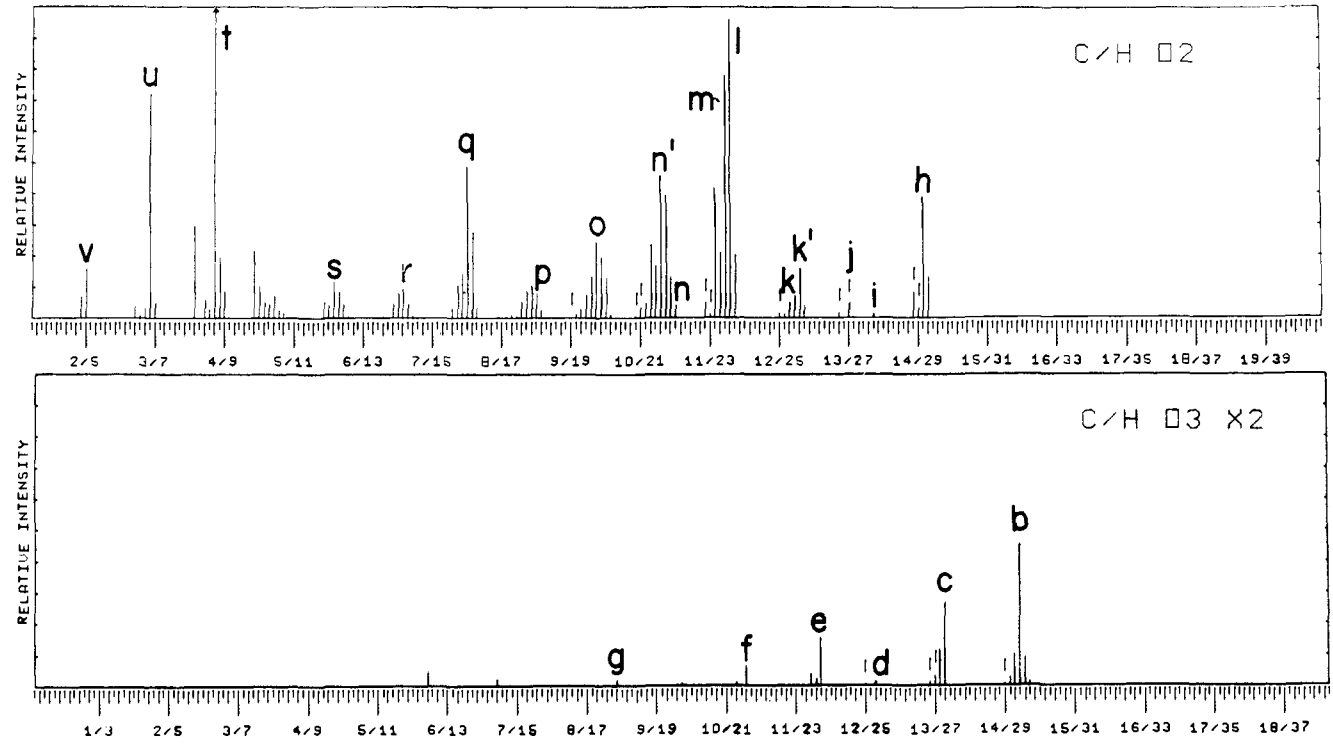


Figure 5. Partial high-resolution mass spectrum of dihydrogaillardin (**9**).

hydrogaillardin (**9**), desacetyldihydrogaillardin (**11**), epoxydihydrogaillardin (**12**), and ketone **14**. The complete high-resolution heteroatomic plots[24] of compounds **8**, **11**, and **14** are shown in Figures 1–3; Figures 4 and 5 show partial high-resolution spectra (the O_2- and O_3-containing fragments) of gaillardin (**6**) and dihydrogail-

(24) The technique of heteroatomic plotting has been developed in our laboratories to provide a reasonable format for the presentation of high-resolution mass spectra. The method is a variation and extension of the "element map" concept proposed by Biemann.[25] The exact mass and composition data from our calculations are sorted according to heteroatom content and each resulting group of data is plotted as a line spectrum. The horizontal scale is divided into major divisions corresponding to the positions and carbon/hydrogen ratios on the line drawing where *saturated fragment* ions would fall. Fragments containing fewer hydrogens than the saturated analog thus lie *below* the major divisions, and this number of hydrogens can be found simply by counting down. For a more detailed explanation including the other types of information that can be gained employing this technique, see A. L. Burlingame and D. H. Smith, in preparation.

(25) K. Biemann, P. Pommer, and D. M. Desiderio, *Tetrahedron Letters*, 1725 (1964).

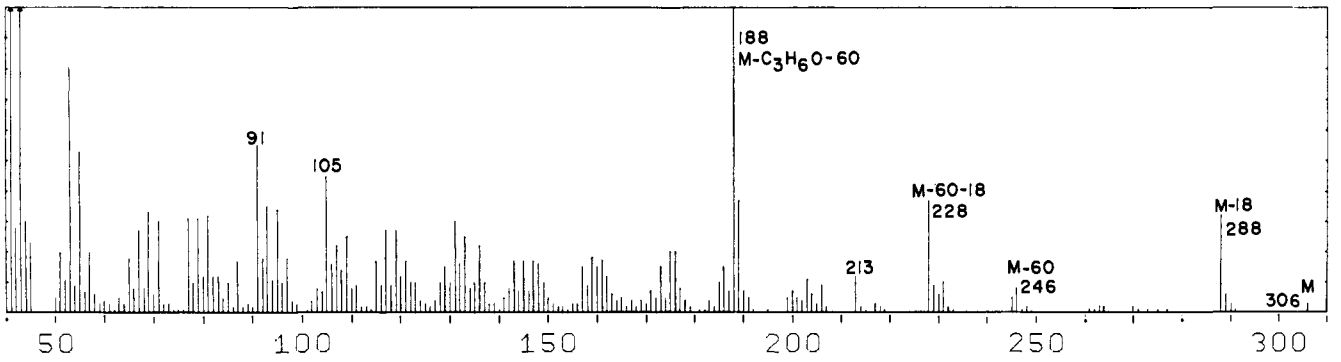


Figure 6. Low-resolution mass spectrum of gaillardin (6).

lardin (9), respectively. Figure 6 represents the conventional low-resolution mass spectrum of gaillardin (6).

We discuss in the following primarily some aspects of the mass spectra of the desacetyl derivatives **8** and **11**. These compounds differ from each other only in that the exocyclic C-11,13 double bond of the lactone is saturated in **11**, thus providing a convenient handle for the identification of certain fragments. For instance, most peaks in the O_2 category of the spectra of **8** and **11** (Figures 1 and 2) are shifted by two mass units (2 H) in the spectrum of **11** when compared to their positions in the spectrum of **8**, an indication that the ions corresponding to these peaks have retained the γ-lactone ring (or at least carbon atoms 11 and 13). In the spectra shown, ions (or peaks) to which we assign the same structural unit (disregarding the difference in simple substituents and in the C-11,13 bond) are given the same label, *i.e.*, a in Figure 1 corresponds to a in Figure 2, except for its shift by two mass units. Ions of somewhat different composition (and structure) but arising presumably by essentially a similar pathway are given primed letters. Suggested mechanisms are illustrated in terms of the saturated derivative (**11**) exclusively.

The details of mechanism and structure cannot be specified from our data, but, as will be illustrated in a few cases, those ionic decompositions which are of obvious significance in terms of the structure of the molecule can usually be rationalized on the basis of common fragmentation reactions (such as cleavages α to hydroxyl functions and subsequent hydrogen-transfer reactions[26,27]) typical for model systems of lesser complexity.

Discussion of the Spectra

I. Simple Elimination of Functionalities. In all spectra, elimination of functional groupings is quite pronounced, a fact which permits the identification of the important functionalities attached to the basic skeleton. The important fragmentations of this type are eliminations of methyl radicals (a, $C_{14}H_{19}O_4$) and of the elements of water (b, $C_{15}H_{20}O_3$), the latter apparently involving both hydroxyl groups. The acetates (**6**, **9**, Figures 4 and 5) predominately eliminate acetic acid from the molecular ion. Other peaks in the spectra can be accounted for by sequential loss of various substituents (*e.g.*, c, h, and j). Peaks i, w, and x could be rationalized by including the elimination of CO in these pathways. Expulsion of CO_2 appears to be a minor fragmentation process. Elimination of the entire lactone ring has been observed for other sesquiterpene systems,[19,20] but for the gaillardin group this pathway is of minor importance. Peak y ($C_{12}H_{15}O$), which does not shift in the dihydro derivative, corresponds to loss of the lactone ring from fragment b; further elimination of water leads to a hydrocarbon fragment yy. The three-carbon moiety lost appears (diprotonated) as ion u ($C_3H_6O_2$), and this sequence may be used to deduce the presence of a γ-lactone grouping. Corroborating evidence is provided by the spectrum of the methoxy derivative where this peak appears as a fragment of composition $C_4H_8O_3$.

II. Fragmentation of Ring A. Cleavages of bonds in the five-membered ring with elimination of two-, three-, or four-carbon fragments containing one or both oxygen functions lead to some of the most prominent peaks of the spectra of gaillardin derivatives, and permit certain reasonable deductions concerning the substitution pattern in ring A.

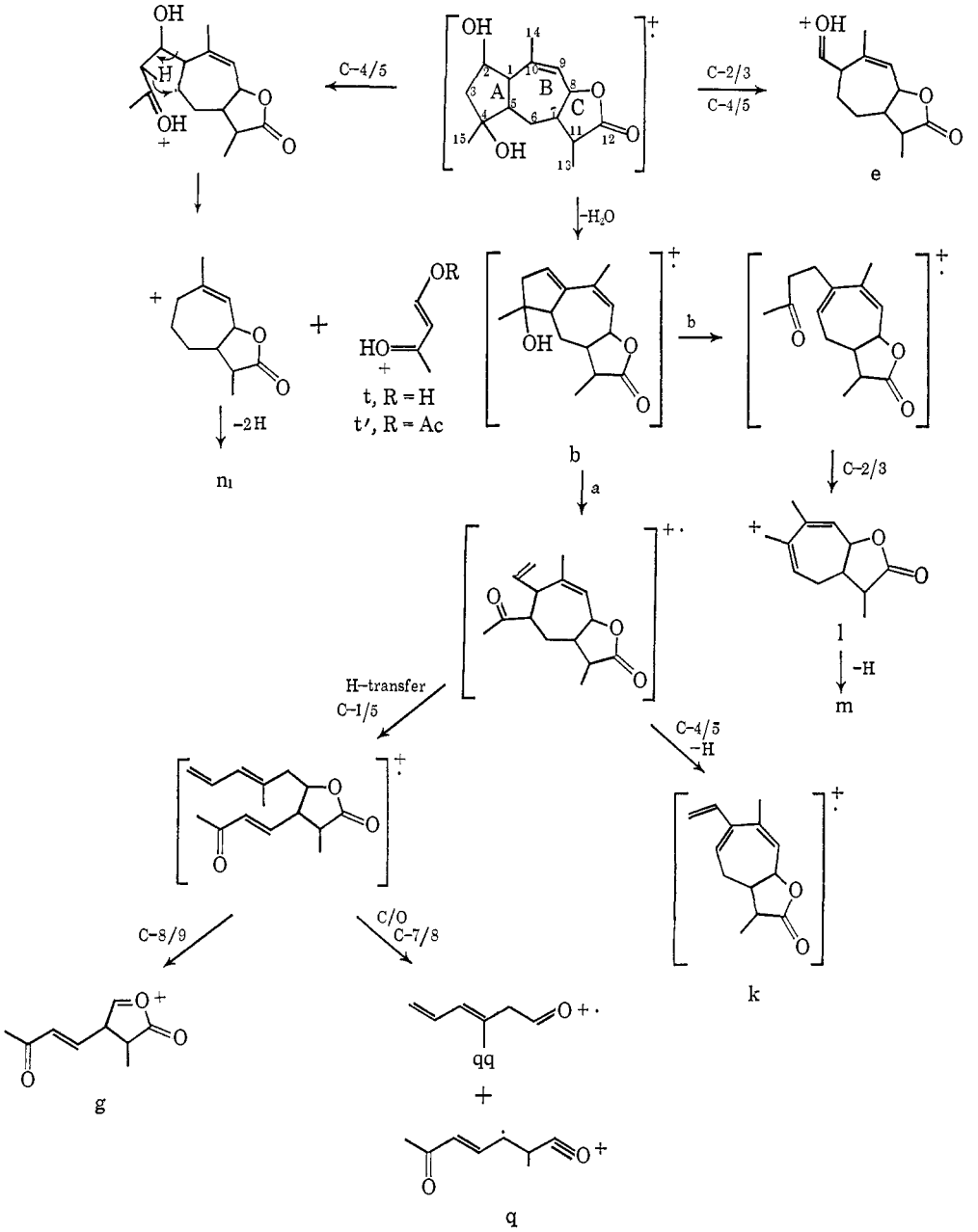
For example, formation of ions d ($C_{13}H_{15}O_3$) and e ($C_{12}H_{16}O_3$, M − C_3H_6O), by loss of two and three carbon atoms with one oxygen substituent, is in excellent agreement with the substitution pattern exhibited by ring A. Appearance of fragments of composition $C_{14}H_{16}O_4$ and $C_{14}H_{18}O_4$ (M − C_3H_6O in each case) in the spectra of **6** and **9**, respectively, seems to demonstrate that indeed carbon atoms 3, 4, and 15 are involved in the decomposition leading to e (compounds **6** and **9** also have peaks at M − C_3H_6O − CH_2CO; see Figures 4 and 5). The spectrum of ketone **14**, not unexpectedly, shows a very prominent ion of composition $C_{12}H_{15}O_3$ (e′).

Ions k ($C_{13}H_{16}O_2$), l ($C_{12}H_{15}O_2$), and m ($C_{12}H_{14}O_2$) involve elimination of two and three carbon atoms, respectively, together with both oxygen atoms. An M − H_2O fragment (b) might be considered the common intermediate from which all three ions arise by simple rearrangement of the C-4 hydroxyl hydrogen atom to the homoallylic C-1,2 double bond.[23,28,29]

As shown in Scheme I, path a could lead to ion k, whereas the alternate route of this hydrogen rearrangement (path b) explains two of the most abundant ions of the two oxygen-containing peaks, namely, fragments l ($C_{12}H_{15}O_2$) and m ($C_{12}H_{14}O_2$). The spectrum (Figure

(26) P. Natalis, *Bull. Soc. Chim. Belges*, **69**, 224 (1961); *Bull. Soc. Roy. Sci. Liege*, **31**, 790 (1962).
(27) H. Budzikiewicz, Z. Pelah, and C. Djerassi, *Monatsh. Chem.*, **95**, 185 (1964).
(28) J. A. Gilpin, *J. Chem. Phys.*, **28**, 521 (1958).
(29) H. E. Audier, H. Felkin, M. Fetizon, and W. Vetter, *Bull. Soc. Chim. France*, 3236 (1965).

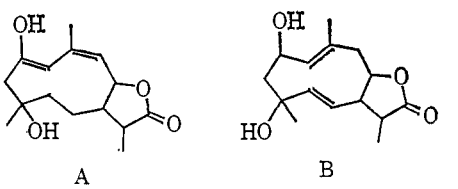
Scheme I



3) of ketone **14** seems to agree with these postulates since here these mechanisms cannot apply, and the peaks corresponding to loss of two or three carbons together with two oxygens are not very prominent.

A complex group of peaks of composition $C_{11}H_{11-14}O_2$ in the spectrum of **8** (Figure 1) and $C_{11}H_{13-16}O_2$ for **11** (Figure 2) must involve loss of the two oxygen functions in ring A, together with four carbons. The mechanism illustrated for the genesis of n_1 follows the pattern expected from the study of model systems.[26,27] The moiety expelled in the decomposition appears in the mass spectrum as an intense peak of composition $C_4H_7O_2$ (t). In the spectra of the acetates **6** and **9**, the same peak is observed, but one also notes a fragment of composition $C_6H_9O_3$ (t'), apparently the corresponding acetyl derivative of t. It is evident that these fragmentations of the five-membered ring are in excellent agreement with the assigned structure and provide good corroborating evidence for a 1,3-hydroxyl substitution pattern.

III. More Complex Fragmentations. Several peaks in the O_3 category of Figures 1 and 2 require loss of four, five, and six carbon atoms with only one oxygen function (*e.g.*, f, f_1, and g), while peaks o, p, r, and s arise by elimination of five, six, seven, and eight carbon atoms and two oxygen substituents, all of which retain, however, the lactone grouping (or at least C-11 and C-13), as evidenced by their shift in Figure 2. The structure of these ions as well as their genesis remains quite obscure, but it is clear that scission of the C-1,5 bond common to rings A and B is required (in the majority of cases) to satisfy their elemental composition. The mechanistic possibilities now become so



A B

complex that little could be gained by illustrating them in detail without supporting evidence. A brief outline will suffice (see structure A and B, above).

E.g., fragments f ($C_{11}H_{13}O_3$) and f_1 ($C_{10}H_{10}O_3$) may arise by decomposition of an intermediate of type A, by loss of four or five carbon moieties (*e.g.*, carbon atoms 4, 5, 6, or 3, 4, 5, 6, plus substituents). The same intermediate, cleaving the appropriate bonds (with rearrangement and/or loss of hydrogen), would furnish ions r ($C_7H_9O_2$), s ($C_6H_7O_2$), and p ($C_9H_{12}O_2$). Fragment o could be derived by similar routes (*e.g.*, decomposition of intermediate B). In Scheme I we have illustrated a possible genesis of fragment g ($C_9H_9O_3$ for **8**, $C_9H_{11}O_3$ for **11**); it may serve at the same time as an example of possible lactone carbon–oxygen bond cleavages to furnish one- and two-oxygen species (peaks q, $C_8H_{10}O_2$, and qq, $C_7H_{10}O$). Fragments such as aa, bb, or bb′ could be generated by similar acyl cleavages of the lactone ring. These multiple hydrogen-transfer and ring-opening reactions contribute greatly to the complexity of the mass spectra, while at the same time permitting few and rather uncertain structural deductions.

A few references have been made to the spectra of the epoxide **12** and the methoxy derivative **5**. In many instances these follow the pattern outlined. However, the tendency of the methoxy compound **5** to eliminate methoxy and methyl ether radicals (CH_2–O–CH_3) introduces further variations into the fragmentation pattern which would require an extensive separate treatment to elaborate fully. The same is true for compound **12**, where now the epoxide function seems to partake to a large extent in the breakdown processes leading to some variations in the general scheme and complicating the picture still further. The spectra illustrate the sensitivity of the fragmentation pattern of these systems to the substituents on the ring skeleton. These compounds will be discussed in more detail when appropriate labeling experiments limit the possibilities for speculation somewhat.

Experimental Section[30]

Gaillardin (6) and Isogaillardin. The extraction and solvent partition of *Gaillardia pulchella* Foug. have been described previously.[6] The 10% aqueous methanol fraction (13.6 g), containing gaillardin (**6**) and isogaillardin, was first chromatographed on 560 g (5 × 37 cm column) of Woelm neutral alumina packed in chloroform–benzene (50:50). The gaillardin-rich yellow band (2 g) was eluted with chloroform–benzene (70:30). This material was rechromatographed on 150 g of silicic acid (100 mesh, Mallinckrodt) packed in benzene. Elution with mixtures of benzene–chloroform gave a fraction (1.2 g) which on crystallization from benzene–Skellysolve B gave 1.0 g of crystalline gaillardin, mp 199–200° (vac); $[\alpha]^{30}D$ −15° (*c* 1.08, $CHCl_3$); λ_{max}^{EtOH} end absorption at 209 mμ (ε 15,500); $\lambda_{max}^{CHCl_3}$ 2.78, 5.67, 5.78, 6.00, 6.04, 8.15 μ; mass spectrum *m/e* 306, molecular ion.

Anal. Calcd for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.84, 66.88; H, 7.24, 7.29.

A second sesquiterpene lactone of slightly lower R_f was isolated (0.35 g) from the same column. This compound was crystallized from benzene–Skellysolve B, giving 0.3 g of isogaillardin, mp 140–141° (vac); $[\alpha]^{27}D$ +113° (*c* 1.21, $CHCl_3$); λ_{max}^{EtOH} high intensity end absorption, 211 mμ (ε 13,300); $\lambda_{max}^{CHCl_3}$ 2.90, 5.69, 5.78, 6.00, 6.09, 7.8–8.1 μ; mass spectrum *m/e* 306, molecular ion.

Anal. Calcd for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.49; H, 7.23.

Dehydrogenation of Gaillardin. Chamazulene (7). Gaillardin (100 mg) was thoroughly mixed with 30% palladium on charcoal (100 mg) and heated in a glass tube in a sublimation block at 295–300° for 20 min. The blue liquid which condensed on the sides of the tube was dissolved in Skellysolve B and chromatographed on a column of Merck acid-washed alumina (5 g). Elution with Skellysolve B gave 1.5 mg of a blue oil which was dissolved in methanol and treated with a solution of 1.5 mg of trinitrobenzene in methanol, giving a crystalline TNB adduct, mp 131–132°. The melting point was undepressed by authentic chamazulene[31] TNB and the ultraviolet spectra of the free azulenes were superimposable.

Dihydrogaillardin (9). a. Catalytic Hydrogenation. A solution of gaillardin (**6**, 105 mg) in methanol was hydrogenated with 55 mg of presaturated 5% palladium on charcoal as catalyst at atmospheric pressure and room temperature. When 1 mole equiv of hydrogen had been absorbed the hydrogenation was stopped. The mixture was filtered and evaporated to give 104 mg of product which was purified by chromatography on a silicic acid–Celite (3:1) (4 g) column (eluent, benzene–chloroform). A homogeneous material (53 mg, one spot tlc) was separated and crystallized from acetone–Skellysolve B to give 45 mg of dihydrogaillardin (**9**), mp 200–201° (vac); $[\alpha]^{27}D$ +48° (*c* 0.89, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ 2.88, 5.63, 5.76, 6.03, 8.02 μ; mass spectrum *m/e* 308, molecular ion.

Anal. Calcd for $C_{17}H_{24}O_5$: C, 66.21; H, 7.85. Found: C, 66.57; H, 7.73.

b. Reduction with Sodium Borohydride. A solution of sodium borohydride (260 mg) in methanol (15 ml) was added to gaillardin (260 mg) in methanol (15 ml) and the solution was stirred at room temperature for 1.5 hr. This solution was poured over 120 ml of ice–water, acidified to pH 1, and extracted with chloroform. The chloroform layer was dried over anhydrous sodium sulfate, filtered, and concentrated to a colorless glass (223 mg). Crystallization from acetone–Skellysolve B gave 220 mg of homogeneous dihydrogaillardin. Recrystallization from the same solvents gave a material identical with the product from catalytic hydrogenation.

Dihydroepoxygaillardin (12). A solution of *m*-chloroperbenzoic acid (58 mg) and dihydrogaillardin (56 mg) in chloroform was refluxed for 2 hr and evaporated to dryness, and the residue was filtered through a column of Merck acid-washed alumina (5 g) packed in benzene. The fractions eluted by benzene–chloroform mixtures and chloroform were combined on the basis of tlc and evaporated, and the residue was crystallized from benzene–Skellysolve B to give 41 mg of crystalline epoxide, mp 172–173° (vac); $[\alpha]^{21}D$ +55° (*c* 1.04, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ 2.88, 5.62, 5.78, 8.04 μ.

Anal. Calcd for $C_{17}H_{24}O_6$: C, 62.95; H, 7.46. Found: C, 62.98; H, 7.56.

Desacetyldihydrogaillardin (11). A solution of dihydrogaillardin (**9**, 100 mg) in 20% aqueous methanol (10 ml) containing KOH (100 mg) was allowed to stand at room temperature overnight. The solution was concentrated, diluted with ice–water, acidified to pH 1, and extracted with chloroform. The chloroform layer was washed with a saturated sodium bicarbonate solution and water, dried over anhydrous sodium sulfate, filtered, and evaporated to give 60 mg of syrup. Chromatography on Merck acid-washed alumina (5 g) gave on elution with benzene–chloroform (50:50) 37 mg of homogeneous compound which, on crystallization from benzene, gave 31 mg of **11**, mp 157–158°; $[\alpha]^{28}D$ +24° (*c* 0.71. $CHCl_3$); $\lambda_{max}^{CHCl_3}$ 2.92, 5.66, 6.03 μ.

Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.78; H, 8.45.

Desacetyldihydrodehydrogaillardin (14). A solution of desacetyldihydrogaillardin (**11**, 72 mg) in acetone (5 ml) was cooled

(30) Melting points were determined on a Thomas–Hoover capillary melting point apparatus which had been calibrated with standard samples. Values of $[\alpha]D$ have been approximated to the nearest degree. Ultraviolet absorption spectra were determined on a Beckman Model DK2A recording spectrophotometer. Infrared absorption spectra were determined on a Beckman Model 5A recording spectrophotometer. Nmr spectra were determined on a Varian A-60 spectrometer in deuteriochloroform solution with tetramethylsilane as internal standard. Chemical shifts are recorded in τ values (ppm) [G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958)]. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich. Low-resolution mass spectra were obtained on a C.E.C.-103 instrument, using an ionizing energy of 70 ev and a current of 20 μa. All high-resolution mass spectra were determined employing a Mattauch-Herzog double-focussing instrument (Consolidated Electrodynamics Corporation type 21-110) with photoplate recording. Spectra were run with an ionizing voltage of 70 ev and a current of 150 μa. Direct introduction of the sample was used for both instruments at minimum temperatures necessary to vaporize the sample.

(31) The authentic chamazulene was obtained from a sample of "Chamomile Oil, Blue, Hungarian" kindly supplied by Mr. Hans R. Schmidt, S. B. Penick and Co. (*cf.* ref 11).

in an ice bath and treated with 15 drops of an 8 *N* chromic acid solution (CrO_3 (2.67 g) in concentrated sulfuric acid (2.3 ml) and water (4.0 ml), diluted to 10 ml with water). The solution was kept in the ice bath for 10 min (frequent swirling), then diluted to 100 ml with water containing a small amount of methanol, and extracted with chloroform. The chloroform layer was washed with water, dried over anhydrous sodium sulfate, filtered, and concentrated to give 66 mg of a solid, mp 177–182°. Recrystallization from benzene–Skellysolve B gave 47 mg of the ketone (**14**), mp 184–186°; $[\alpha]^{29}D$ −198° (*c* 0.88, $CHCl_3$); λ_{max}^{EtOH} end absorption at 212 mμ (ε 6050); $\lambda_{max}^{CHCl_3}$ 2.90, 5.66, 5.75, 6.03 μ.

Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.06; H, 7.57.

Dehydration of Desacetyldihydrodehydrogaillardin (14). a. Thionyl Chloride–Pyridine. A solution of **14** (50 mg) in pyridine (5 ml) was treated with thionyl chloride (2 ml) with stirring for 0.5 hr in an ice bath and then 1 hr at room temperature. The reaction mixture was diluted with water and extracted with chloroform. The chloroform layer was washed with dilute acid, saturated sodium bicarbonate solution, and water, dried over anhydrous sodium sulfate, filtered, and concentrated to give 32 mg of syrup which could not be crystallized. The infrared absorption spectrum was consistent with structure **13**; $\lambda_{max}^{CHCl_3}$ 5.62 (s), 5.90 (s), 6.18 (m), 6.02 (w) μ.

b. *p*-Toluenesulfonic Acid. A mixture of **14** (50 mg) and *p*-toluenesulfonic acid (20 mg) in benzene (10 ml) was refluxed for 25 min. The reaction mixture was diluted with water and extracted with chloroform. The chloroform layer was washed with saturated sodium bicarbonate solution and water, dried over anhydrous sodium sulfate, filtered, and concentrated to a syrup. This material was purified by preparative thick layer (1 mm) chromatography (Brinkmann silica gel HF; methanol–chloroform (2:98); bands detected by ultraviolet lamp). Extraction of band II with chloroform gave 40 mg of a homogeneous material which was crystallized from ether–Skellysolve B to give 24 mg of the dienone **15**, mp 146–147°; $[\alpha]^{29}D$ + 37° (*c* 0.683, $CHCl_3$); λ_{max}^{EtOH} 255 mμ (ε 16,600); $\lambda_{max}^{CHCl_3}$ 5.63 (s), 5.95 (s), 6.11 (m), 6.18 (s) μ.

Anal. Calcd for $C_{15}H_{18}O_3$: C, 73.14; H, 7.37. Found: C, 72.97; H, 7.46.

13-Methoxydesacetylgaillardin (5). A solution of gaillardin (**6**, 55 mg) in 20% aqueous methanol (5 ml) containing potassium carbonate (100 mg) was refluxed 1 hr and allowed to stand at room temperature overnight. The solution was concentrated, diluted with ice–water, acidified to pH 1 with dilute hydrochloric acid, and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate, filtered, and evaporated to give 48 mg of syrup containing a mixture of **5** and **8**. Chromatography on Merck acid-washed alumina (5 g) with benzene–chloroform (25:75) gave 22 mg of **5**. Recrystallization from methylene chloride–Skellysolve B gave 10 mg of **5**, mp 151–152°; $\lambda_{max}^{CHCl_3}$ 2.90 (OH, strong), 5.65 μ (lactone carbonyl); mass spectrum *m/e* 296.

Anal. Calcd for $C_{16}H_{24}O_5$: C, 64.83; H, 8.16. Found: C, 65.04; H, 8.27.

Desacetylgaillardin (8). A solution of gaillardin (**6**, 97 mg) in 40% aqueous dioxane (15 ml) containing potassium hydroxide (150 mg) was allowed to stand for 1 day. This solution was concentrated, diluted with water, acidified, and extracted with methylene chloride. The methylene chloride layer was washed with saturated sodium bicarbonate solution and water, dried over anhydrous sodium sulfate, filtered, and evaporated to give 76 mg of syrup. Crystallization from benzene–Skellysolve B gave 20 mg of **8**, mp 148–150°. Chromatography of the mother liquor from crystallization on Merck acid-washed alumina (9 g) gave, on elution with benzene–chloroform (50:50), 35 mg of a syrup containing **8**. Crystallization from benzene–Skellysolve B gave an additional 22 mg of **8**, mp 149–151°; $[\alpha]^{29}D$ −38° (*c* 0.807, $CHCl_3$); λ_{max}^{EtOH} end absorption at 210 mμ (ε 14,600); $\lambda_{max}^{CHCl_3}$ 2.90 (OH strong), 5.66 μ (lactone carbonyl).

Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.12; H, 7.37.

Tetrahydrogaillardin (10). A solution of gaillardin (**6**, 87 mg) in ethyl acetate (20 ml) was hydrogenated in the presence of 10% palladium on charcoal (80 mg) at atmospheric pressure and room temperature. Uptake of hydrogen stopped after approximately 0.5 hr, at which time 2 mole equiv of hydrogen had been absorbed. The suspension was filtered and concentrated to give a syrup (85 mg). Chromatography on Merck acid-washed alumina (10 g) with benzene–chloroform (75:25) gave **10** (55 mg) which could not be crystallized.

Treatment of Gaillardin with Acetic Anhydride–Pyridine. A solution of gaillardin (**6**, 50 mg) in acetic anhydride (2 ml) and dry pyridine (4 ml) was allowed to stand at room temperature for 24 hr. Standard work-up led to recovery of starting material (40 mg).

Action of α-Chymotrypsin on the Diethyl Esters of Fumaric, Maleic, and Acetylenedicarboxylic Acids[1]

Saul G. Cohen, Lucille H. Klee, and Sandra Y. Weinstein

Contribution from the Department of Chemistry, Brandeis University, Waltham, Massachusetts 02154. *Received June 29, 1966*

Abstract: Diethyl maleate is not hydrolyzed, while diethyl fumarate is hydrolyzed by α-chymotrypsin, $k_{cat} = 0.28$ sec^{-1}, $K_{m,app} = 0.023$ *M*. The fumarate shows reactivity a little greater than that of diethyl succinate, and leads to monoethyl fumarate in high yield. Diethyl acetylenedicarboxylate is hydrolyzed by α-chymotrypsin with first-order kinetics, the enzyme becoming inactivated. It is concluded that common substrates for the enzyme, α,β-disubstituted propionates, hydrolyze in a conformation in which the β substituent and the hydrolyzing ester or amide group are transoid. The distance between the entrances to the aryl, ar, and nucleophilic, n, sites of the enzyme is ~3.8 A. The results indicate that the cyclic substrate D-1-keto-3-carbomethoxytetrahydroisoquinoline is hydrolyzed by the enzyme with the carbomethoxyl group in the equatorial conformation.

Small molecule substrates for α-chymotrypsin of varied structure have been studied and information has been obtained about the effects of substituents on the rates and stereospecificity of their enzymic hydrolyses.[2,3] A major objective has been to draw inferences about the size and geometry of the active area of the enzyme, about the interactions between

(1) We are pleased to acknowledge generous support of this work by the Division of Research Grants, National Institutes of Health, GM-04584. This constitutes article XI on the specificity of α-chymotrypsin; for the previous article in this series see S. G. Cohen and S. Y. Weinstein, *J. Am. Chem. Soc.*, **86**, 5326 (1964).

(2) G. H. Hein and C. Niemann, *Proc. Natl. Acad. Sci. U. S.*, 47, 1341 (1961).

(3) S. G. Cohen, J. Crossley, E. Khedouri, R. Zand, and L. H. Klee, *J. Am. Chem. Soc.*, **85**, 1685 (1963).