Structure of the Antibiotic Botryodiplodin—Use of Chemical Ionization Mass Spectrometry in Organic Structure Determination[1]

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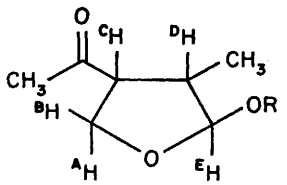
Summary The structure assigned to botryodiplodin is based mainly on physical methods including chemical ionization mass spectrometry used for the first time as a tool in organic structure elucidation.

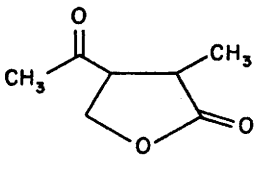
SOME physical, chemical, and biological properties of the compound botryodiplodin were reported by Sen Gupta, Chandran, and Divekar who isolated this antibiotic from *Botryodiplodia theobromae* Pat.[2] We assign structure (I) to botryodiplodin based on physical and chemical evidence.

The i.r. spectrum of botryodiplodin showed ketone carbonyl absorption at 1713 cm.$^{-1}$ and hydroxyl absorption at 3400 cm.$^{-1}$. Acetylation of botryodiplodin with acetic anhydride and anhydrous pyridine afforded a product (II) with no hydroxyl absorption in its i.r. spectrum but with an added carbonyl absorption band at 1735 cm.$^{-1}$, indicating the presence of a saturated ester. Hydrolysis of botryodiplodin acetate (II) with water at room temperature regenerated botryodiplodin.



(I) R = H

(II) R = $COCH_3$



(III)

The electron-impact mass spectrum of botryodiplodin could not be used reliably to determine the molecular weight because the appearance of the spectrum changed with time, the method of sample introduction and the instrument used. Chemical ionization mass spectrometry[3,4] which, by the completion date of this work, had been applied solely to study the behaviour of relatively simple organic molecules introduced *via* a heated inlet system (gold leak) proved to be useful in determining the molecular weight of botryodiplodin. A consolidated Electrodynamics Corporation 21-110B double focussing mass spectrometer was modified to permit its use for both chemical and electron-impact modes of ionization.[5] The chemical ionization mass spectrum of botryodiplodin shown in the Figure was obtained by vaporizing the sample directly into the ion source held at a pressure of 1 torr of methane. The molecular weight of botryodiplodin was deduced to be 144 on the basis of the arguments which follow, even though the molecular ion is absent in the spectrum as is almost always the case in chemical ionization mass spectra.[3,4,6] Reaction of botryodiplodin (I) with CH_5^+ would lead to proton addition on one of three possible oxygen sites and the formation of species with *m/e* 145 $(M + 1)^+$ and, after the loss of the elements of water, *m/e* 127. Hydride ion abstraction by $C_2H_5^+$ would give rise to $(M - 1)^+$ at *m/e* 143. The abundant ions at the high-mass end of the spectrum are thus accounted for. Less abundant peaks occurring at yet higher mass-to-charge ratios confirm the assigned molecular weight and are due to collision-stabilized adducts formed by botryodiplodin and $(C_2H_5)^+$ $[(M + C_2H_5)^+$ at *m/e* 173] or $(C_3H_5)^+$ $[(M + C_3H_5)^+$ at *m/e* 185], or these adducts less the elements of water (*m/e* 155 and 167).

Measurement of the exact masses of all ions in the chemical ionization mass spectrum of botryodiplodin was made to determine the elemental composition of the antibiotic and to facilitate further interpretation of the spectrum. The exact masses (to ± 1 millimass unit) were obtained at a resolving power of *ca.* 1 : 10,000, using the photoplate recording technique[5,7] and the recently developed evaporated silver bromide photographic plate.[8,9] The elemental composition of most ions are shown in the Figure

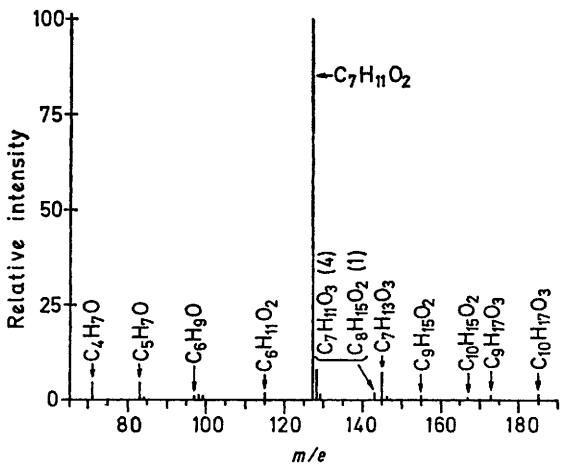


FIGURE. *Chemical ionization mass spectrum of botryodiplodin; source temperature*: 60°.

and permitted assigning to botryodiplodin (I) the elemental composition $C_7H_{12}O_3$ on the basis of the same arguments which were used to deduce its molecular weight.

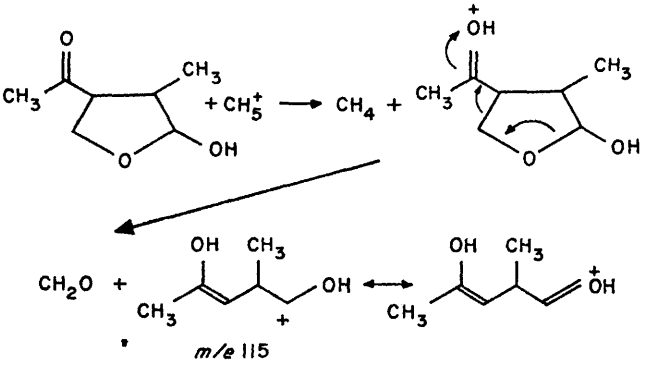
The n.m.r. spectrum of botryodiplodin was complex due to the presence of unequal amounts of two anomers in solution. The anomeric protons appeared as a broad singlet at 5·09 p.p.m. Oxidation of botryodiplodin with silver oxide afforded botryodiplodin lactone which showed no hydroxyl absorption in its i.r. spectrum but carbonyl absorption at 1715 (ketone) and 1777 cm.$^{-1}$ (γ-lactone). No anomeric proton was present in the n.m.r. spectrum of the lactone and no peak (other than isotope peaks) appeared above *m/e* 142 in its electron-impact mass spectrum. At

the outset it was not known whether the molecular ion or a fragment ion of the lactone gave rise to *m/e* 142. That question was readily answered in favour of the former once the molecular weight of botryodiplodin was established by chemical ionization mass spectrometry. These data are compatible with structure (III) for the lactone.

In support of structure (I) for botryodiplodin the 60 MHz n.m.r. spectrum of botryodiplodin acetate (II) shows resonance signals at 2·03 p.p.m. (3H, s,† CH_3CO_2), 2·20 p.p.m. (3H, s, CH_3COC), 0·93 p.p.m. (3H, d, CH_3CH, J 7·0 Hz), 5·85 p.p.m. (1H, s, ^{E}H), 2·65 p.p.m. (1H, quin, ^{D}H), 3·51 p.p.m. (1H, q,‡ ^{C}H) and 4·16 p.p.m. (2H, quin,§ $^{A}HC^{B}H$). Irradiation of the methyl doublet at 0·93 p.p.m. resulted in the collapse of the quintet at 2·65 p.p.m. to a doublet with J_{CD} 7·0 Hz. Thus, the chemical shifts and coupling constants observed allow the relative placement of the methylene, C-acetyl, and methyl groups as shown in (II). The anomeric proton at 5·85 p.p.m. shows a sharp singlet, indicating the presence of a single anomer in solution.

The 7·0 Hz coupling constant between ^{C}H and ^{D}H, and their presence in a five-membered ring suggests a *cis*-relationship. This conclusion is subject to the uncertainty of assigning stereochemistry to substituted tetrahydrofurans based on coupling constants.[10]

The potential usefulness of chemical ionization mass spectrometry is not limited to determining molecular weights or elemental compositions. An equally important aspect is the interpretation of fragmentation occurring subsequently to ion–molecule reactions in the gas phase, thus providing an additional technique in structure elucidation. For example, *m/e* 115 may arise as follows:



The minor component of the doublet of *m/e* 143 which has one-fourth the intensity of $(M - 1)^+$ has an elemental composition $C_8H_{15}O_2$ (Figure). It may arise *via* the same mechanism starting from the adduct $(M + C_2H_5)^+$.

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† s = singlet; d = doublet; q = quartet; and quin = quintet.
‡ At 100 MHz this broad quartet is discernible as two overlapping triplets.
§ Resolved into two triplets at 100 MHz which show that $J_{AB} = J_{AC} = J_{BC} = 8·6$ Hz by first-order interpretation.

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