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# CRYSTAL STRUCTURE OF SPICATIN HYDROBROMIDE. REVISION OF THE STRUCTURE OF SPICATIN.

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Spicatin, $C_{27}H_{32}O_{10}$, a gummy cytotoxic[1] sesquiterpene lactone of the guaianolide type from two Liatris species, has been provisionally formulated as 1 on the basis of spectroscopic data and chemical transformations; the alternative structure 2, in which the location of the cis-sarracinoyl and cis-acetylsarracinoyl side chains is interchanged could not be excluded with certainty. In order to settle this ambiguity, a single crystal X-ray analysis of spicatin hydrobromide was undertaken.

Spicatin hydrobromide (3), $C_{27}H_{33}O_{10}Br$, m.p. 95-98° (dec.), prepared by treatment of spicatin with 48% HBr in ethanol, forms monoclinic crystals of space group $P2_1$. Crystal data are: $a$ = 10.415 (5), $b$ = 16.093 (2), $c$ = 8.122 (3) Å, $\beta$ = 96.97 (2)°; $z$ = 2. X-ray intensity data were collected with a Phillips PW 1100 computer-controlled diffractometer. 2621 reflexions were measured out to $\theta$ = 70°. 2401 of these were considered to be observed ($\sigma(I)/I \leq 0.25$) and were used in the subsequent refinements.

The structure was solved by a combination of heavy-atom and direct methods.[3] The $x$ and $z$ coordinates of the bromine atom position were refined by least-squares to an $R$ value of 0.374. The structure factors were converted to normalized /$E$/'s using an approximate absolute scale and average temperature factor determined by Wilson's method. The atomic position of the bromine atom was used to calculate structure factors. The phases of 28 reflexions with /$Fc$/ $\geq$ 0.5 /$Fo$/, where /$Fc$/ and /$Fo$/ are calculated and observed structure

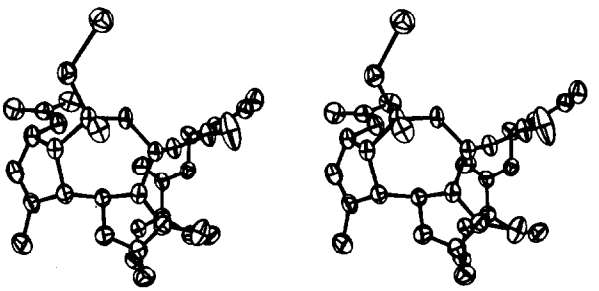
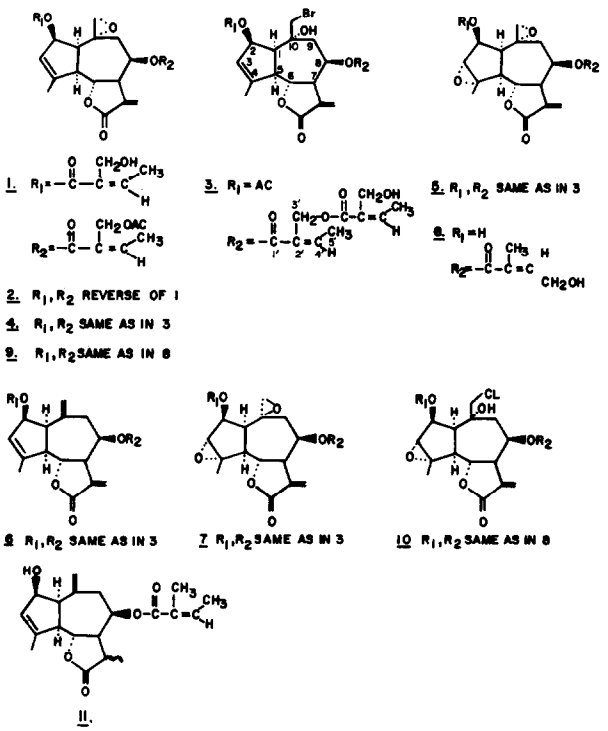


Fig. 1. Stereoscopic view of spicatin hydrobromide (3).



factor magnitudes respectively, were used as starting phases together with the phases of two reflexions as variables. These phases were extended and refined by use of a modified version of the MULTAN direct phase determination procedure.[4] The calculated E map unvoidably contained false symmetry planes through the bromine atom. Considerable effort was involved in separation of true and false peaks. Besides, only part of the molecule was phased by the bromine atom contribution. However, a fragment of chain $R_2$ (3) was successively located which partly suppressed the false symmetry. After repeated use of Fourier syntheses the structure determination converged to a single molecule, (3). With anisotropic thermal parameters the non-hydrogen atoms were refined to an R value of 0.053. At this point a difference Fourier synthesis was calculated and 28 of the hydrogen atoms were located. Four of the non-observed hydrogen atom positions were calculated. The hydrogen atoms were included in the refinement with fixed positional and isotropic thermal parameters and the structure was refined to R = 0.038. A stereoscopic view of spicatin hydrobromide (3) is shown in Fig. 1.

The X-ray analysis establishes that the structure of spicatin must be modified from 1 to 4; the only difference being the nature of the acyl groups on C-2 and C-8. Thus, an acetate group is located at C-2 in spicatin (4), whereas the ester side chain attached to C-8 comprises two cis-sarracenoyl units, one of which is esterified on C-3' of the other. It follows that the spicatin conversion products, epoxyspicatin, deoxyspicatin and -10(14)-β-epoxyspicatin should be reformulated as 5, 6 and 7 respectively. The X-ray study, however, confirms the structures and stereochemistries previously deduced for graminiliatrin (8), deoxygraminiliatrin (9) and graminichlorin (10), since these compounds have been correlated with spicatin (4).

The reduction of spicatin with zinc copper couple carried out in the presence of hydrochloric acid[2] affects the side chains and requires comment. The reaction, which results in the formation of the monoester 11, involves reduction of the conjugated lactone system accompanied by deoxygenation of the epoxide group. The ester side chain on C-2 is hydrolyzed, whereas that on C-8 undergoes hydrogenolysis to a tigloyl residue. This result was considered to favour formula 1 for spicatin rather than formula 2, since acetate is a better leaving group than hydroxyl.[2] However, it is now evident that the reaction involved hydrolytic loss of the acetate substituent on C-2 and hydrogenolysis of the allylic C-3' oxygen bond of the C-10 ester side chain on C-8 with formation of the $C_5$-residue of 11.

Spicatin (4) is the second sesquiterpene lactone encountered so far, which possesses

a $C_{10}$ ester side chain made up of two $C_5$ acyl units. The first such compound, the germacranolide provincialin, was also isolated from a Liatris species; one of the $C_5$ units of its $C_{10}$ ester side chain is a cis-sarracinoyl grouping.[5]

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