

THE STRUCTURE AND STEREOCHEMISTRY OF SPATHELIN, A NEW SECO-RING A TETRANORTRITERPENE FROM *SPATHELIA SORBIFOLIA*

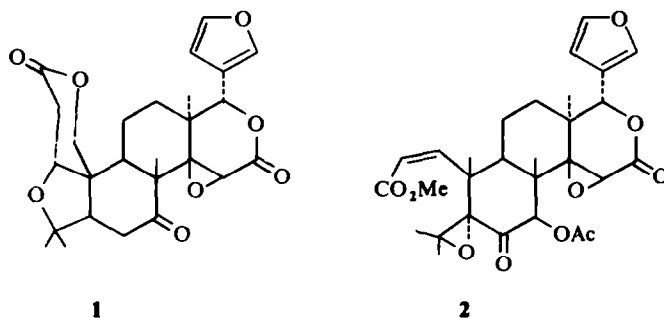
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Abstract—Spathelin is shown to have structure 2 by physical and chemical methods, including a correlation with isoobacucnic acid.

THE GENUS *Spathelia* has been placed in the Simaroubaceae by Bentham and Hooker, whereas Engler classifies it as Rutaceae.¹ Since several genera of the Rutaceae but none of the Simaroubaceae have yielded tetranortriterpenes of the limonin (1) type (limonoids),² the isolation of these compounds from a *Spathelia* species would be of taxonomic significance. To this end we have examined *Spathelia sorbifolia* and have reported the isolation of several new chromones.³ We now report the isolation, and proof of structure, of a new seco-ring A limonoid from this plant.⁴ Benzene extraction of the roots and chromatography of the extract on alumina gave spathelin, C₂₉H₃₄O₁₀, m.p. 178.5–180°, [α]_D –7°, which we show to have structure 2.



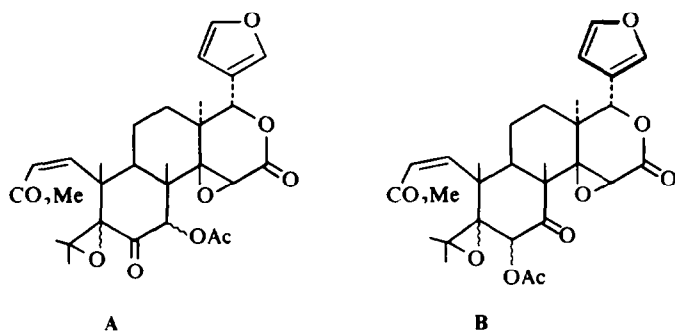
The IR spectrum has the typical bands for a β -substituted furan (3080, 1500 877 cm⁻¹) and a series of carbonyl bands at 1748, 1742, 1727 and 1712 cm⁻¹ together with olefinic absorption at 1634 cm⁻¹. There was no absorption attributable to OH stretching vibration. There were two maxima in the UV at 206 and 298 nm (ϵ 11,500 and 38). The latter band can be associated with an isolated carbonyl group while the high extinction coefficient of the low wavelength band, which is somewhat twice that expected of a furan system, suggests that another chromophore is superimposed on that of the β -substituted furan.

In view of the many correlations which have been established, the NMR spectrum

of a limonoid can be especially revealing⁵ and when this is combined with biogenetic arguments,² it can lead to a powerful probe of molecular structure. Such was the case with spathelin. A β -substituted furan was confirmed by multiplets at δ 7.43 (2H) and 6.38 (1H). There were five tertiary Me's as singlets at δ 0.85, 1.23 (6H), 1.27 and 1.33, an acetate and an OMe group. A sharp singlet (1H) at δ 3.83 is characteristic of H-15 in a ring D epoxy lactone. This system is confirmed by a slightly broadened singlet (1H) at δ 5.50 attributable to H-17. A conjugated *cis*-disubstituted double bond gave rise to an AB pattern (J_{AB} 12.5 Hz) at δ 6.22 and 5.83 and could reasonably be associated as an α,β -unsaturated methyl ester to account both for the intensity of the low wavelength band in the UV and the OMe group already observed. There is only one further band in the low field region of the spectrum, a singlet at δ 6.25 which can be ascribed to a proton at the base of an acetate. The position is at lower field than that normally expected and reference to this point will be made later. Its singlet nature would indicate that the adjacent carbon atoms are fully substituted.

Confirmation for some of the functional groups suggested by the spectral data came from lactone titration of spathelin which showed the presence of three hydrolysable residues. Nine of the oxygen atoms have been assigned and it is clear that, given the molecular formula and in the absence of OH groups, spathelin must be bicarbocyclic.

All the limonoids of the Rutaceae, so far known,² are oxidatively modified by Baeyer-Villiger type oxidation in rings A and D. Taken with the above data this then suggests that there are two alternative expressions, A and B, which could be written for spathelin.

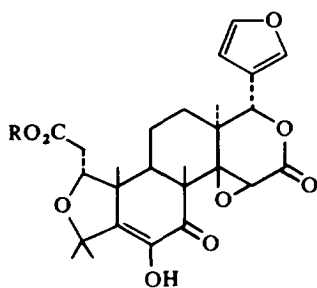


The placing of the acetate group and the 4, 5-epoxide is dictated by (a) the number of Me singlets and (b) the singlet nature and chemical shift of the proton at the base of the acetate. The biogenesis of limonoids leading to hydroxylation at C-7 in a euphane precursor requires that the OH group be α -oriented.² However, with a carbonyl group at C-6, (as in A), there is the possibility of epimerization at C-7.

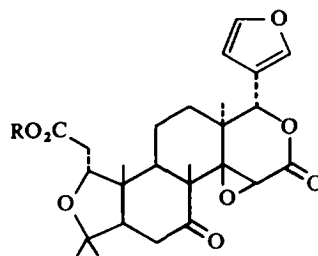
Of decisive importance in establishing the structure of spathelin was the product obtained on treatment with base. A yellow colour was immediately produced on the addition of aqueous alkali to a solution of spathelin in MeOH. Acidification of the mixture gave an acid, $C_{26}H_{30}O_9$, m.p. 288–291° which, on the basis of the evidence below, is unequivocally defined as 3.

A maximum in the UV at 276 nm (ϵ 8600) which undergoes a bathochromic shift

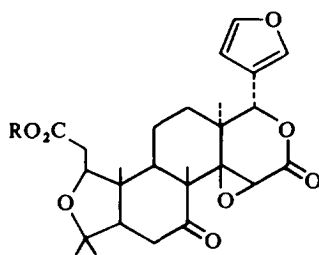
on the addition of alkali is characteristic of a diosphenol moiety. The β -substituted furan is associated with a maximum at 205 nm (ϵ 5400) and the decrease in intensity as compared with the precursor, suggests the loss from spathelin of a chromophore which was contributing to the absorption at this wavelength. In the IR, there were absorptions for hydrogen bonded OH and for carbonyl bands at 1745 (lactone), 1712 (acid) and 1686 and 1664 cm^{-1} (diosphenol). Treatment with CH_2N_2 gave the amorphous methyl ester (4).



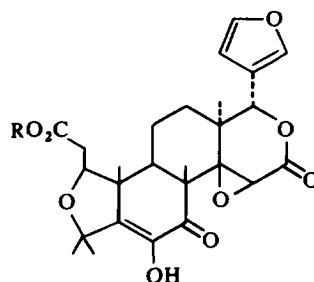
3 R = H
4 R = Me



5 R = Me
10 R = H



6 R = Me
7 R = H



8 R = H
9 R = Me

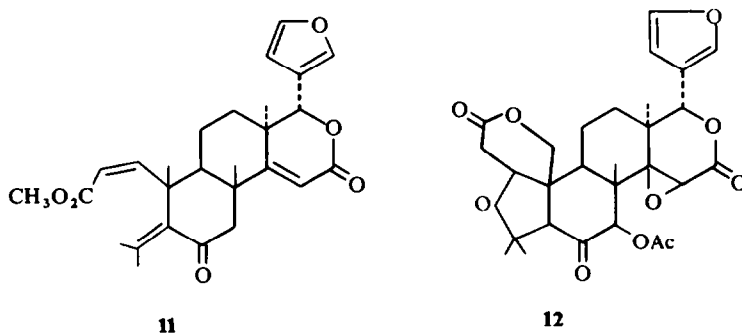
The NMR spectrum of the methyl ester (4) showed singlets at δ 4.13 and 5.46 for H-15 and H-17 respectively. A broadened singlet at δ 6.28, disappearing after exchange with D_2O , can be assigned to the OH group of the diosphenol. No olefinic protons were present but there was now a quartet centred at δ 4.35 (J 8.5, 6.5 Hz) assigned to a β -oriented proton at C-1 on the basis of comparison with model compounds. In veprisone (5) and its derivatives,⁶ H-1 appears as a well resolved quartet at δ 4.30 (J 10, 5 Hz), while in the methyl isoabacunoate (6) which is epimeric at C-1, it is shifted upfield to 3.94 and now appears as a triplet (J 5.8 Hz).

Confirmation of the structure (3) for the diosphenol was achieved by direct correlation with isoabacunoic acid (7). Oxygenation of isoabacunoic acid in *t*-BuOH in the presence of *t*-BuOK has been reported to give the diosphenol (8).⁷ This was converted with CH_2N_2 to the methyl ester 9 whose spectral data are in full accord with the structure. The IR spectrum of the methyl ester 9 was similar but not identical with that of the methyl ester 4 derived from spathelin. The NMR spectrum, in particular, showed a triplet at ϵ 3.97 (J 6.0 Hz) in agreement with the α -configuration of H-1.

This ester was recovered unchanged from treatment with NaOMe in MeOH. However, when ester **4** obtained from spathelin was treated under the same conditions, the product was identical to that obtained from isoobacunoic acid. The epimerization at C-1 under these conditions is to be expected: epi-isoobacunoic acid (**10**) is converted to isoobacunoic acid (**7**) under basic conditions.⁸

The generation of diosphenol **3** on base treatment of spathelin can be accommodated on either of the alternative structures A and B *via* the enolate ion. A decision in favour of A was made from the result of CrCl₂ reduction of spathelin. After four days, a mixture of products resulted from which the major compound could be isolated by prep. TLC. This compound, C₂₇H₃₂O₆, m.p. 167–170°, is formulated as the *cisoid* enone (**11**) on the basis of spectral evidence. Conjugated carbonyl absorption is present in the UV [λ_{\max} 250 nm (ϵ 7700)] and IR (ν_{\max} 1661 cm⁻¹) spectra. The NMR spectrum shows retention of the β -substituted furan and the α,β -unsaturated methyl ester. There are now three tertiary Me's and two vinylic Me's appear as singlets at δ 1.71 and 1.94. In agreement with the formulation as a ring D unsaturated lactone, H-17 is a broadened singlet at δ 5.03 while H-15 appears as a sharp singlet at 5.96. This value is in accord with those found in odoratin (δ 5.89)⁹ and carapin (δ 5.78)¹⁰ where the position corresponding to C-7 is a methylene group. It is known that a carbonyl group at C-7 exerts a marked downfield shift on H-15.⁵ Spathelin was recovered from treatment with acid similar to that obtaining in the CrCl₂ reduction, thus ruling out the possibility of an isomerization prior to the reduction.

The correlation of spathelin with isoobacunoic acid (**7**) has established the configuration at all asymmetric centres with the exception of C-5 and C-7. The arrangement as written in structure **2** was deduced from spectral considerations.



The chemical shift of H-15 in ring D epoxy lactones is dependent on the substituent at C-7.⁵ With an α -acetate in this position H-15 normally is found near δ 3.50. In spathelin, this proton resonates at 3.83 and has an identical shift as in rutaevin acetate (**12**)¹¹ which, on the basis of other evidence, has been formulated with a 7β -acetate. A comparison of the half height widths of the H-15 ($W_{\frac{1}{2}}$ 0.9 Hz) and H-7 ($W_{\frac{1}{2}}$ 1.8 Hz) singlets indicate that H-7 is coupled at long range to other protons—most likely those of the C-8 Me. The W arrangement of bonds which is best for this type of interaction can only be achieved when H-7 is axial. Support for this comes from an examination of the C-7 methylene protons in the *cisoid* enone (**11**). These appear as an AB pattern in which the lower field doublet attributable to H-7 (axial) is significantly broadened relative to that at higher field which is due to H-7 (equatorial).

The configuration of the 4,5-epoxide in spathelin follows from its anisotropic effect on the axial C-7 proton. In spathelin this proton shows a pronounced downfield shift (δ 6.25) when compared with the position in rutaevin acetate (5.65). Such an effect can only be explained if the epoxide oxygen is near to H-7 and hence α .¹²

Limonoids have only been isolated from the Rutaceae and Meliaceae. Thus, the isolation of spathelin (2) from *Spathelia sorbifolia* supports its inclusion in the Rutaceae rather than the Simaroubaceae. However, the observation² that seco-ring A limonoids are peculiar to the Rutaceae has been invalidated by two recent reports¹³ of such compounds from the Meliaceae.

EXPERIMENTAL

M.p.'s were determined on a Kofler hot stage apparatus and are uncorrected. UV data are for EtOH solns, IR spectra for Nujol mulls and rotations in CHCl_3 solns unless stated otherwise. NMR spectra were recorded in CDCl_3 with TMS as internal reference.

All evaporations were carried out on a rotavapor under reduced pressure.

Isolation of Spathelin. The finely powdered root of *Spathelia sorbifolia* L. (3.2 Kg) was percolated with C_6H_6 (3:1). Removal of solvent *in vacuo* left a viscous oil (34.8 g) which was chromatographed on alumina (grade III). The elution of the limonoid was monitored by TLC and Ehrlich's reagent. Crystallization of the Ehrlich's positive fraction gave spathelin (2) as prisms from MeOH, 1.1 g, m.p. 178.5–180°, $[\alpha]_{\text{D}} - 7^\circ$, λ_{max} 206 and 298 nm (ϵ 11,500 and 38), ν_{max} 3080, 1748, 1742, 1727, 1712, 1634, 1500, 877 cm^{-1} , δ 7.43 (2H) and 6.38 (1H) (β -substituted furan), 6.22 and 5.83 (1H each, AB pattern, J_{AB} 12.5 Hz, H-1 and H-2), 6.25 (1H, s, H-7), 5.50 (1H, s, H-17), 3.83 (1H, s, H-15), 3.72 (3H, OMe), 3.48 (3H, MeOH of crystallization, absent from a dried sample), 2.28 (3H, OAc), 1.33, 1.27, 1.23 (2), 0.85 (CMe). (Found: C, 63.1; H, 6.6; O, 30.5. $\text{C}_{29}\text{H}_{34}\text{O}_{10}$. CH_3OH requires: C, 62.7; H, 6.7; O, 30.6%).

Base treatment of spathelin. The diosphenolic acid (3). A mixture of spathelin (101 mg) and 0.1N NaOH aq (15 ml) was heated on a water bath until the solid had dissolved. After cooling, titration of the soln against 0.1N H_2SO_4 using phenolphthalein as indicator showed the presence of three acidic groups. The product, obtained after further acidification and extraction into CHCl_3 , crystallized in prisms from EtOAc to give the acid (3), 64 mg, m.p. 288–291°, λ_{max} 205 and 276 nm (ϵ 5400 and 8600) shifting on addition of base to 332 nm (ϵ 5950), ν_{max} 3350, 3106, 2793, 1745, 1712, 1686, 1664, 1499, 877 cm^{-1} . (Found: C, 64.1; H, 6.3. $\text{C}_{26}\text{H}_{30}\text{O}_9$ requires: C, 64.2; H, 6.2%).

The methyl ester (4). This was obtained as an amorphous solid from acid 3 on treatment with CH_2N_2 in the usual way. It had $[\alpha]_{\text{D}} - 61^\circ$, λ_{max} 205 and 275 nm (ϵ 6700 and 7500) shifting on addition of base to 320 nm (ϵ 4000), ν_{max} 3344, 1739, 1681, 1658, 1495, 877 cm^{-1} , δ 7.45 (2H) and 6.39 (1H) (β -substituted furan), 6.28 (1H, OH, exchanges with D_2O), 5.46 (1H, s, H-17), 4.35 (1H, q, J 8.5 and 6.5 Hz, H-1), 4.13 (1H, s, H-15), 1.64, 1.55, 1.49, 1.11 and 1.08 (CMe). (Found: C, 64.7; H, 6.45. $\text{C}_{27}\text{H}_{32}\text{O}_9$ requires: C, 64.8; H, 6.4%).

The diosphenol (8) from isoobacumic acid. A soln of isoobacumic acid (119 mg) and t-BuOK in t-BuOH (15 ml) was shaken under oxygen for 2 hr. After dilution with water and acidification with 6N HCl, the product was extracted into CHCl_3 . The CHCl_3 extract was washed with 5% aq. NaHCO_3 . The product obtained on acidification of the bicarbonate extract was the amorphous diosphenol (8), 91 mg, λ_{max} 206 and 275 nm (ϵ 6100 and 5900) shifting on addition of alkali to 325 nm (ϵ 3050), ν_{max} 3378, 2597, 1739, 1712, 1685, 1656, 1499, 877 cm^{-1} .

The methyl ester (9) was obtained on treatment with CH_2N_2 in the usual way. It was amorphous but homogeneous on TLC and had $[\alpha]_{\text{D}} - 104^\circ$, λ_{max} 206 and 275 nm (ϵ 6000 and 7000) shifting on addition of base to 330 nm (ϵ 4000), ν_{max} 3378, 1745, 1684, 1658, 1499, 877 cm^{-1} , δ 7.45 (2H) and 6.40 (1H) (β -substituted furan), 5.49 (1H, s, H-17), 4.17 (1H, s, H-15), 3.97 (1H, t, J 6.0 Hz, H-1), 3.72 (3H, OMe), 1.55, 1.47, 1.29, 1.17 and 1.11 (CMe). (Found: C, 65.2; H, 6.6. $\text{C}_{27}\text{H}_{32}\text{O}_9$ requires: C, 64.8; H, 6.4%).

Epimerization of the methyl ester (4). A soln of the ester (4) (60 mg) and NaOMe (500 mg) in MeOH (8 ml) was heated under reflux for 90 min. The product, obtained by dilution with water and extraction into EtOAc, was identical with the diosphenol methyl ester (9) derived from isoobacumic acid by TLC, rotation and spectral comparison.

The ester (9) was recovered unchanged when subjected to the conditions described above.

Chromium(II) chloride reduction of spathelin. An excess of CrCl_2 (from $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$, 2.7 g) in N HCl (10 ml) was added to a soln of spathelin (100 mg) in acetone (50 ml) and the mixture stored under CO_2 for 4 days. The product (75 mg), recovered with CHCl_3 , was purified by prep. TLC. The major compound was the *enone* (11) which crystallized from EtOH in prisms, 35 mg, m.p. 167–170°, λ_{max} 211 and 250 nm (ϵ 15,700 and 7700), ν_{max} 1721, 1661, 1499, 877 cm^{-1} , δ 7.48 (2H) and 6.44 (1H) (β -substituted furan), 6.31 and 5.74 (1H each, AB system, J_{AB} 12.5 Hz, H-1 and H-2) 5.96 (1H, s, H-15), 5.03 (1H, s, H-17), 3.60 (3H, OMe), 3.09 and 2.76 (1H each, AB system, J_{AB} 13.5 Hz, $W_{\frac{1}{2}}$ for inner lines = 2.7 and 1.4 Hz respectively, 2 \times H-7), 1.94 and 1.71 (vinylic Me), 1.44, 1.28 and 1.21 (CMe). (Found: C, 71.7; H, 7.4. $\text{C}_{27}\text{H}_{32}\text{O}_6$ requires: C, 71.7; H, 7.1%).

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