

IDENTIFICATION OF 4-PHENYL AND 4-ALKYLCUMARINS IN MAMMEA AMERICANA L., MAMMEA AFRICANA G.DON AND CALOPHYLLUM INOPHYLLUM BY GAS CHROMATOGRAPHY - MASS SPECTROMETRY

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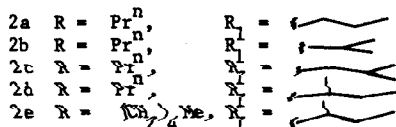
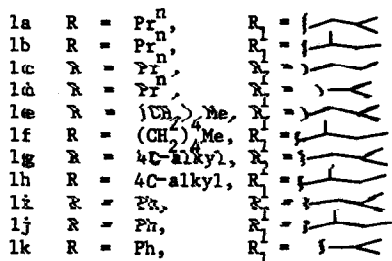
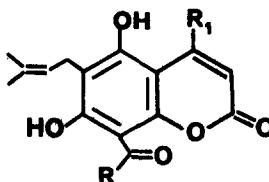
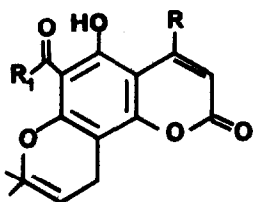
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Although mass spectrometry has been used extensively in the structural elucidation of natural oxygen heterocycles, gas chromatography - mass spectrometry (g.c.-m.s.) has received little attention in the investigation of compounds of this type.¹ We now find that coumarins, chromones, acylphloroglucinols and rotenoids are amenable to g.c.-m.s. investigation, without derivatization. The technique has been applied in the study of some of the coumarin containing fractions obtained from three members of the Guttiferae family, resulting in the identification of the five new natural coumarins (1c, 1d, 1e, 1f, 2b), partial formulations for seven coumarins, and evidence for the presence of a number of known coumarins not previously identified in the particular plant.

The light petroleum extract of the seeds of Mammea americana L. contains a large number of coumarins, exhibiting wide structural variation.² Four 2,2-dimethylpyranocoumarins (1a,b,i,j) have been identified from this source,^{3,4} and we have detected an additional three pairs of isomeric coumarins (M.W. 398, 384, 356) and one coumarin (M.W. 390) of this type, by g.c.-m.s. examination. The mass spectra of the eleven compounds all had as base peak, the ion formed by loss of a methyl radical from the molecular ion thus indicating the presence of a 2,2-dimethylchromen.⁵ Fragmentation of the molecular ion by cleavage α -to the carbonyl group was indicative of the nature of the acyl group; 2-methylpropionyl and butyryl groups yielding M-43 ions and 2-methylbutyryl and 3-methylbutyryl groups yielding ions at M-57.⁶ Further differentiation was made from the intensities of these ions, 2-methylpropionyl and

2-methylbutyryl groups yielding more intense ions than butyryl and 3-methylbutyryl groups. On the basis of these fragmentations, the coumarins were assigned structures (1a, b, i, j, e, f, g, h, c, d and k) or 5,7-dioxygenated structures isomeric with these.* Comparisons with synthetic material^{4,7} (m.s.-g.c. retention times and g.c. co-injections) confirmed the formulations (1a-f, i-k)** for nine of these coumarins.



G.c.-m.s. of a sample of the coumarin (2a) isolated from Mammea americana L.,⁶ indicated that a second isomeric coumarin was present, and it was assigned structure (2b) on the basis of its mass spectrum (m/e (% abundance) 358 (27), 315 (75), 303 (11), 259 (100), (231 (12)).

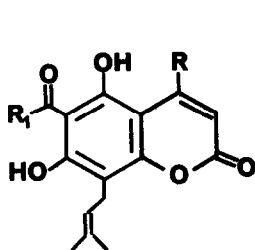
Previous investigators have isolated nine coumarins from the bark of Mammea africana G.Don.⁹ Using g.c.-m.s., we have commenced an examination of an extract from this source resulting in confirmation of the presence of coumarins (1b, j), (2d), (3a,b,c) and the identification of additional coumarins (1a,c), (1e or f), (1i), (2c,e) in the extract.




Calophyllolide (4a), cis and trans-inophyllolide (5a) and the coumarin (5b) have been isolated from the seeds¹⁰ and leaves¹¹ of Calophyllum inophyllum L. G.c.-m.s. examination of a chromatographic fraction obtained from a seed extract of Calophyllum showed the presence of

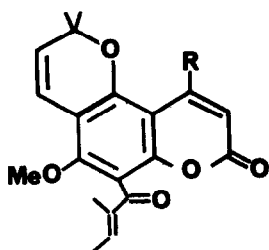
* On the basis of mass spectra we are unable to differentiate between this formulation, the isomeric linear 2,2-dimethylpyranocoumarin and the isomeric 8-acyl formulations.

** Mesuagin (1k) was first reported in the seed oil of Mesua ferrea.⁸

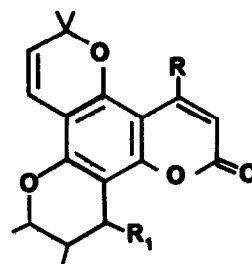
seven coumarins of M.W. 416, 416, 396, 386, 382, 366 and 352. In their mass spectra each compound had an ion at (M-15) as base peak, indicating the presence of an annulated 2,2-dimethylpyrano group,⁵ and the compounds of M.W. 416, 416, 396, 382, also had significant peaks at m/e 83 and m/e 55 consistent with formulations (4a, b, c) or the isomeric structures (6a,b,c) or (7a,b,c). The compounds of M.W.416 were shown by g.c. comparison, to be calophyllolide (4a) and apetalolide (6a).¹² Mass spectra of this extract, obtained by temperature programming the direct insertion probe confirmed the presence of the compounds of M.W. 416, 396 and 382, but the other molecular ions were replaced by ions at m/e 404, 384, 370, suggesting that dehydration of these compounds had occurred during the g.c.-m.s.-investigation. Further examination of the mass spectra (m/e 404, 389, 333, 305; m/e 384, 369, 351, 285; m/e 370, 355, 337, 299, 271) of these compounds suggested formulations (5b,c,d) or (8a,b,c). Costatolide (5c, R = Prⁿ)¹³ and the coumarin (5b)¹¹ have been isolated from C.costatum and C.inophyllum respectively, however direct g.c.comparisons of these compounds with our material have not been possible because of the dehydration occurring during gas chromatography.



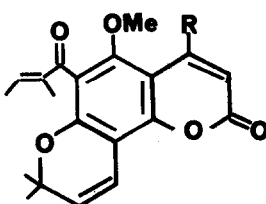
3a R = Ph, R₁ = 
 3b R = Ph, R₁ = 
 3c R = Prⁿ, R₁ = 



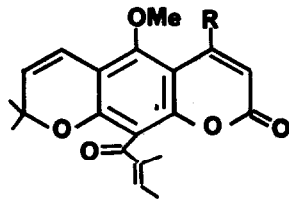
4a, R = Ph
 4b, R = 4C alkyl
 4c, R = 3C alkyl



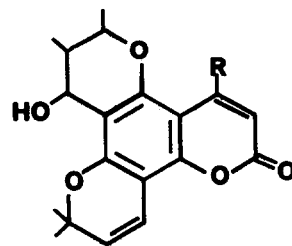
5a R = Ph, R₁ = =O
 5b R = Ph, R₁ = OH
 5c R = 4C alkyl, R₁ = OH
 5d R = 3C alkyl, R₁ = OH



6a R = Ph
 6b R = 4C alkyl
 6c R = 3C alkyl



7a R = Ph
 7b R = 4C alkyl
 7c R = 3C alkyl



8a R = Ph
 8b R = 4C alkyl
 8c R = 3C alkyl

Mass spectra were obtained in line diagram and mass list form with background subtracted and corrected for changes in total ionization during scan with the aid of a Varian 620i computer on line to the mass spectrometer. Unresolved peaks on the gas chromatographs were deconvoluted by repetitive scanning over the unresolved region and using the computer to obtain plots of mass against time for the molecular ions and fragment ions. Glass columns (3% OV 1 or 3% OV 17 on Gas Chrom Q) were used in the g.c. and the coupling to the mass spectrometer was all glass, via a two stage Watson-Biemann separator.

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