

## LACTONIC CONSTITUENTS OF *PRANGOS PABULARIA* LINDL (UMBELLIFERAE)

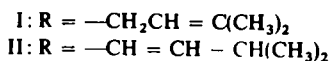
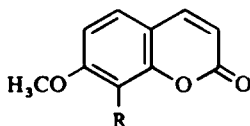
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**Abstract**—The structures of pabularinone (III) and pabulenol (IV), two new, minor furocoumarin constituents of *Prangos pabularia* Lindl., have been elucidated from spectroscopic studies and chemical reactions and subsequently confirmed by their syntheses and correlation with compounds of known structures.

*Prangos pabularia* Lindl. (family: Umbelliferae, tribe: Symyrnieae) is well known for producing a large number of coumarins with isoprenoid units disposed in multifarious ways. This plant has been found to be relatively rich in secondary metabolic products, namely furocoumarins and terpenoids.<sup>1</sup> It is interesting that no angular furocoumarins have as yet been isolated from this umbelliferous species. Osthol (I) and its isomer (II) are the two non-furanolactones so far obtained.

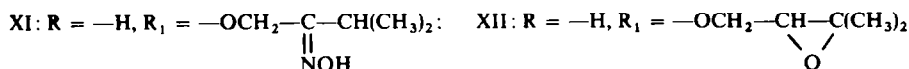
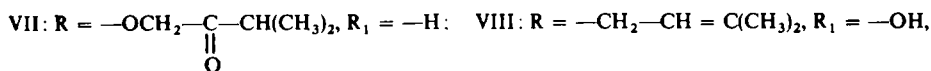
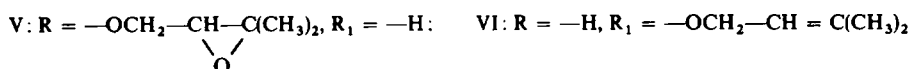
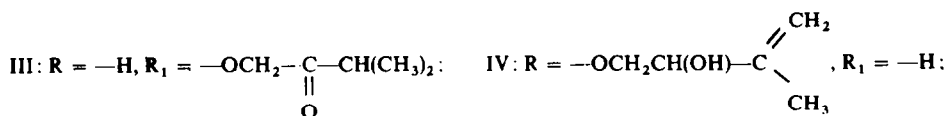
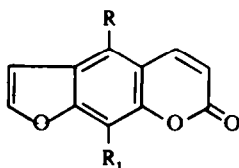


Of the seventy two *Prangos* species known till now only *P. pabularia* Lindl. is indigenous to India. This tall perennial herb is held in high repute for the medicinal value of its roots and fruits.<sup>1</sup> Exhaustive studies on the petrol extract of the roots of this rich coumarin source culminated in the isolation and structure elaboration of two new linear furocoumarins pabularinone (III) and pabulenol (IV) in addition to oxypeucedanin (V), osthol (I), imperatorin (VI), isooxypeucedanin (VII) and allo-imperatorin (VIII). Isooxypeucedanin (VII) had been isolated for the first time from the genus *Prangos*. Moreover, before the identity of this compound was established we studied its NMR and mass spectra which have not been previously reported.<sup>2, 3</sup>

Part of the work dealing with the characterization of pabularinone (III) and pabulenol (IV) has been published.<sup>4, 5</sup> The present communication is concerned with the details of our previous work and the synthesis of pabulenol (IV).

Extraction of the dried and powdered roots of *P. pabularia* with petrol (b.p. 60–80°) followed by column chromatography over silica gel afforded two new coumarins pabularinone (III) and pabulenol (IV). Pabularinone (III)  $\text{C}_{16}\text{H}_{14}\text{O}_5$  ( $\text{M}^+$  286), m.p. 131–132°,  $[\alpha]_D^{25} = 0^\circ$  (EtOH) exhibited UV absorption  $[\lambda_{\text{max}}^{\text{C}_2\text{H}_5\text{OH}} 219, 250 \text{ and } 300 \text{ nm}]$

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(log  $\epsilon$ : 4.39, 4.38 and 4.09 respectively)] very similar to those of 8-oxygenated linear furocoumarins.<sup>6</sup> The IR spectrum indicated the presence of a coumarin lactone (1736 cm<sup>-1</sup>), an unconjugated CO function (1712 cm<sup>-1</sup>), an ether linkage (1214 and 1011 cm<sup>-1</sup>) and benzofuran moiety (1058 cm<sup>-1</sup>). The weak absorption at 1618 cm<sup>-1</sup> suggested the possibility of the compound being an 8-substituted linear furocoumarin.<sup>7</sup> The 60 MHz NMR spectrum studied in CDCl<sub>3</sub> showed two pairs of doublets, one at  $\delta$  6.35 and  $\delta$  7.80 ( $J = 10$  Hz) attributed to the C-3 and C-4 protons of the coumarin nucleus while the second pair of signals at  $\delta$  6.80 and  $\delta$  7.65 ( $J = 2$  Hz) confirmed the presence of the benzofuran moiety. In the low field region the one proton singlet at  $\delta$  7.35 indicated the presence of a single aromatic proton.

On heating with glacial acetic acid containing a few drops of concentrated sulphuric acid pabularinone (III) lost a C<sub>5</sub>-unit (-C<sub>5</sub>H<sub>5</sub>O) furnishing a phenolic compound, xanthotoxol (IX), C<sub>11</sub>H<sub>6</sub>O<sub>4</sub> (M<sup>+</sup> 202), m.p. 248-250°, identified as its methyl ether, xanthotoxin (X), C<sub>12</sub>H<sub>8</sub>O<sub>4</sub> (M<sup>+</sup> 216), m.p. 147-148°. The concomitant expulsion of a C<sub>5</sub>-unit with the formation of xanthotoxol (IX) proved pabularinone (III) to be an 8-prenoxy-psoralen derivative. The conspicuous absence of any long range coupling ( $J = 1$  Hz) of the aromatic proton with that of the  $\beta$ -furan<sup>8,9</sup> and the chemical shift of the C-4 proton,  $\delta$  7.80, compared to those of C-5 prenylated furocoumarins,  $\delta$  8.40 in case of isooxypeucedanin (VII) and  $\delta$  8.04 for alloimperatorin (VIII), studied during this investigation, further corroborated this view.<sup>10</sup> The NMR spectrum of isooxypeucedanin (VII) was closely related to pabularinone (III) except for the  $\beta$ -furan proton in the former which underwent long range coupling with the C-8 aromatic proton at  $\delta$  7.18 ( $J = 1$  Hz), a characteristic feature of bergaptol derivatives.<sup>8,9</sup>

On treatment with hydroxylamine hydrochloride in the presence of anhydrous

sodium acetate<sup>11</sup> pabularinone formed a monooxime (XI), C<sub>16</sub>H<sub>15</sub>NO<sub>5</sub> (M<sup>+</sup> 301), m.p. 142–144°, thereby confirming the presence of a CO function in the prenyl side chain. The oxime lacked the broad band at 1712–1736 cm<sup>-1</sup> present in the parent coumarin but showed a new absorption at 3237 cm<sup>-1</sup> attributable to the oxime hydroxyl. Even in this neutral medium pabularinone underwent cleavage to xanthotoxol (IX) which was the major product obtained during oximation.

The structural pattern of the prenyl moiety has been settled by the NMR spectrum. A six proton doublet at  $\delta$  1.20 ( $J = 7$  Hz) and an incompletely resolved septet centred around  $\delta$  3.00 revealed the presence of a gem-dimethyl group and an adjacent methine proton ( $-\text{CH}(\text{CH}_3)_2$ ). The two proton singlet at  $\delta$  5.20 has been assigned to the methylene protons (C') ( $-\text{OCH}_2-\overset{\text{O}}{\underset{\text{O}}{\text{C}}}-\text{CH}(\text{CH}_3)_2$ ), the down field shift of which is

justified due to the combined diamagnetic effects of the aromatic nucleus and the carbonyl group.

Based on these spectral and chemical evidences the structure of pabularinone was settled as III. This structure received unequivocal support from the 70 e.v. mass spectrum of the coumarin which rendered diagnostic peaks at  $m/e$  286 (M<sup>+</sup>; 43%), 216 (24%), 215 (61%), 202 (12%), 201 (6%), 186 (10%), 185 (8%), 173 (6%), 145 (4%), 71 (38%) and 43 (100%). It may be pertinent to mention here that the 70 e.v. mass spectrum of isooxypeucedanin (VII) was very similar to that of its C-8 isomer (III) and exhibited significant peaks at  $m/e$  286 (M<sup>+</sup>; 92%), 216 (16%), 215 (25%), 202 (11%), 201 (19%), 187 (21%), 186 (10%), 185 (7%), 173 (8%), 145 (7%), 89 (4%), 71 (38%) and 43 (100%).

An independent evidence in favour of structure III for this 8-prenoxy linear furocoumarin was secured from its partial synthesis from imperatorin (VI), C<sub>16</sub>H<sub>14</sub>O<sub>4</sub> (M<sup>+</sup> 270), m.p. 99–100°, isolated in abundance during this investigation. The latter on epoxidation with perbenzoic acid at room temperature afforded oxyimperatorin (XII), C<sub>16</sub>H<sub>14</sub>O<sub>5</sub> (M<sup>+</sup> 286), m.p. 112–114°, which on treatment with BF<sub>3</sub>·etherate at room temperature furnished pabularinone. This was isolated by preparative TLC. The reaction product, thus obtained, was found to be identical with natural pabularinone from mixture m.p. determination, co-TLC behaviour and superimposable IR spectra.

In a similar manner isooxypeucedanin (VII) could be synthesized from oxypeucedanin (V). Thus, this Lewis acid catalysed transformation opened up an efficient method (yield: > 50%) for the synthesis of prenyl or prenoxy ketones from the corresponding epoxides compared to the older method of using 10–20% mineral acid (yield: 6%).

The second coumarin, pabulenol (IV), C<sub>16</sub>H<sub>14</sub>O<sub>5</sub> (M<sup>+</sup> 286.0852), m.p. 134–135°, [ $\alpha$ ]<sub>D</sub><sup>25</sup> = -3.8° (EtOH) has been isolated in 0.005% yield from the root portion of *P. pabularia*. Its UV spectrum in the region 225–340 nm [ $\lambda_{\text{max}}^{\text{C}_2\text{H}_5\text{OH}}$ : 249.5, 267 and 308 nm (log  $\epsilon$ : 4.17, 4.12 and 4.02 respectively)] was typical of a linear furocoumarin bearing similarity with bergapten<sup>6, 12</sup> while the IR spectrum revealed a hydroxyl function (3497 cm<sup>-1</sup>), a lactone carbonyl (1709 cm<sup>-1</sup>), a benzofuran moiety (1075 cm<sup>-1</sup>), an aromatic ether (1214, 1105 cm<sup>-1</sup>) and a terminal methylene (893 cm<sup>-1</sup>). Negative ferric chloride test coupled with the absence of any bathochromic shift in the UV absorption maxima in alkali pointed to the alcoholic nature of the OH

function. The IR spectrum of pabulenol was akin to those of 5-substituted furocoumarins e.g. oxypeucedanin (V) ( $1602, 1623 \text{ cm}^{-1}$ ) in the region  $1600\text{--}1650 \text{ cm}^{-1}$ . Within this frequency range these compounds exhibited twin bands unlike the 8-substituted compounds which exhibited a weak absorption between  $1620\text{--}1625 \text{ cm}^{-1}$ .<sup>7</sup> Pabulenol exhibited the significant twin bands at  $1603$  and  $1634 \text{ cm}^{-1}$ .

The 100 MHz NMR spectrum in  $d_6$ -DMSO confirmed the presence of those functionalities already inferred from the UV and IR absorption spectra. The characteristic coumarin doublets appeared at  $\delta 6.25$  and  $\delta 8.25$  ( $J = 10 \text{ Hz}$ ) while the  $\alpha$ -furan proton resonated as a doublet at  $\delta 7.95$  ( $J = 2 \text{ Hz}$ ). The low field value of the C-4 proton suggested pabulenol to be a C-5 substituted coumarin.<sup>10</sup> The  $\beta$ -furan proton merged with the aromatic proton resulting in a finely split two proton signal at  $\delta 7.24$ .

The nature of the prenyl moiety has been confirmed from the NMR spectrum supported by chemical reactions. The three proton multiplet centred around  $\delta 4.35$  has been assigned to the two methylene and the adjacent methine protons

( $-\text{OCH}_2-\overset{\diagup}{\underset{\diagdown}{\text{C}}}\text{H}$ ) and the signal at  $\delta 5.36$  (1H, d,  $J = 4 \text{ Hz}$ ), disappearing on deutera-

tion, to the aliphatic hydroxyl. The three proton singlet at  $\delta 1.71$  has been attributed to the vinylic Me, the assignment of which is justified by anisotropic and deshielding effects of the neighbouring vinyl and OH functions. The terminal vinyl protons appeared at  $\delta 5.06$  and  $\delta 4.86$ , the latter showing a barely visible long range coupling ( $J = 1 \text{ Hz}$ ).

The presence of a single, allylic and secondary OH function was corroborated from the formation of pabulenol monoacetate (XIII),  $\text{C}_{18}\text{H}_{16}\text{O}_6$  ( $M^+ 328$ ), m.p.  $119\text{--}119.5^\circ$ , on treatment with acetic anhydride and pyridine at room temperature. The OH band was replaced by peaks at  $1739$  and  $1235 \text{ cm}^{-1}$  attributable to the acetate function in the IR spectrum. The acetoxy function exhibited a tall three proton singlet at  $\delta 2.12$  while the carbinol methine appeared unusually downfield at  $\delta 5.65$  (1H, t,  $J = 5 \text{ Hz}$ ). The  $\beta$ -furan resonated as a doublet at  $\delta 6.96$  ( $J = 3 \text{ Hz}$ ). Further splitting of this signal by long range coupling with the C-8 aromatic proton ( $J = 1 \text{ Hz}$ ) proved a 5-substituted skeleton for pabulenol.<sup>8,9</sup>

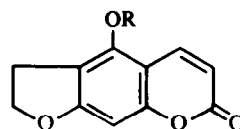
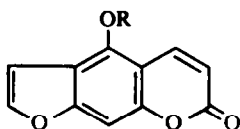
Acid catalysed rearrangement using 10% sulphuric acid of pabulenol afforded isooxypeucedanin (VII),  $\text{C}_{16}\text{H}_{14}\text{O}_5$  ( $M^+ 286$ ), m.p.  $142\text{--}144^\circ$ , of known structure. This reaction is typical of systems possessing an allylic OH function, a vinylic methyl and a terminal methylene group ( $-\text{CH}(\text{OH})-\text{C}(\text{CH}_3)=\text{CH}_2$ ). Oxypeucedanin hydrate (XIV),  $\text{C}_{16}\text{H}_{16}\text{O}_6$  ( $M^+ 304$ ), m.p.  $129\text{--}130^\circ$ , was also obtained. The identity of these two reaction products was established by comparison with authentic samples. In the IR spectrum of isooxypeucedanin (VII) the peaks for OH and terminal methylene were missing while the CO band was considerably enhanced (broad band at  $1709\text{--}1727 \text{ cm}^{-1}$ ).

Hydrogenation of pabulenol in neutral medium (95% aldehyde-free ethanol) yielded a complex mixture from which small amounts of the tetrahydro derivative (XV),  $\text{C}_{16}\text{H}_{18}\text{O}_5$  ( $M^+ 290$ ) and dihydrobergaptol (XVI),  $\text{C}_{11}\text{H}_8\text{O}_4$  ( $M^+ 204$ ) could be retrieved by preparative TLC. These compounds were characterized from their mass spectral analyses. The UV absorption spectrum of dihydrobergaptol ( $\lambda_{\text{max}}^{\text{C}_2\text{H}_5\text{OH}}$  267.5, 290 and 332 nm) lacked the characteristic benzofuran absorption in the 240–250 nm region thereby indicating reduction of the furan double bond in preference to

the  $\alpha$ -pyrone system. This reductive cleavage of pabulenol to dihydrobergaptol even in neutral medium confirmed the presence of the ether linkage in the molecule.

In the 70 e.v. mass spectrum of tetrahydropabulenol (XV) the base peak at  $m/e$  204 was obtained by loss of 86 amu from the molecular ion ( $M^+$  290). This was confirmed by the presence of a metastable peak at  $m/e$  143.5 ( $M^+$  290  $\xrightarrow{m/e\ 143.5}$   $m/e$  204). The very loss of 86 amu in this reduced product compared to 84 amu in the parent coumarin yielding the base peak  $m/e$  202 ( $M^+$  286  $\xrightarrow{m/e\ 142.6}$   $m/e$  202) proved the presence of an unsaturation in the five carbon unit side chain of pabulenol.

All these spectral and chemical data can readily be explained on the basis of structure (IV) for this 5-prenoxy linear furocoumarin.



IV: R =  $-\text{CH}_2-\text{CH}(\text{OH})-\text{C}(\text{CH}_3)=\text{CH}_2$ :

XIII: R =  $-\text{CH}_2-\text{CH}(\text{OCOCH}_3)-\text{C}(\text{CH}_3)=\text{CH}_2$ :

XIV: R =  $-\text{CH}_2-\text{CH}(\text{OH})-\text{C}(\text{CH}_3)_2-\text{OH}$

XV: R =  $-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}(\text{CH}_3)_2$

XVI: R =  $-\text{H}$

This structure was further confirmed by the synthesis of the compound from oxy-peucedanin (V) by treatment with  $\text{BF}_3 \cdot \text{etherate}$  in dioxan at room temperature. The starting material (V) was isolated in good yield (1.26%) from the roots of *P. pabularia*. The reaction mixture on preparative thin layer chromatography afforded an appreciable quantity of isooxypeucedanin (VII) and a solid, m.p. 120–122°. The latter exhibited a single spot on TLC using various solvent systems. However, from NMR and mass spectral analyses the second product was found to be a mixture of pabulenol

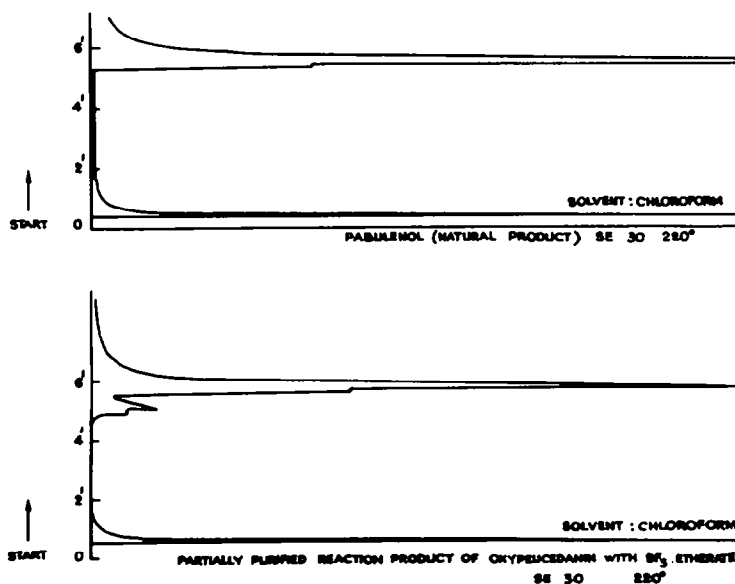


FIG 1

( $M^+$  286) and a minor component ( $M^+$  306). Pabulenol was separated from this mixture on an LKB MS gas liquid chromatograph using a silicone SE 30 column, the temperature being maintained at 220°. Helium was used as the carrier gas. An identical experiment was run with the pure natural product. The minor component showed a retention time of 5 minutes while the major component was found to be identical with natural pabulenol both in retention time of 5 min – 44 sec (Fig 1) and mass spectra.

The 70 e.v. mass spectrum of pabulenol rendered diagnostic peaks at  $m/e$  286 ( $M^+$ ; 19%), 202 (100%), 174 (52%), 146 (8%), 145 (13%), 90 (10%), 89 (17%), 43 (13%) and 41 (28%) all of which are consistent with structure IV for the coumarin. The base peak at  $m/e$  202 (202.0263 by high resolution corresponding to the formula  $C_{11}H_6O_4$ ; calculated: 202.0266) was obtained by the loss of a prenyl unit from the molecular ion  $M^+$  286.0852 corresponding to  $C_{16}H_{14}O_5$  (calculated: 286.0841). This was supported by the metastable peak at  $m/e$  142.6. The nature of the prenyl moiety was further confirmed from the 70 e.v. mass spectrum of pabulenol acetate (XIII). In addition to the molecular ion peak at  $m/e$  328 (15%), the generation of the fragment ions  $m/e$  127 (54%) and 85 (19%) demanded the presence of the side chain  $—CH_2—CH(OCOCH_3)—C(CH_3)=CH_2$  in pabulenol acetate and hence of  $—CH_2—CH(OH)—C(CH_3)=CH_2$  in pabulenol.

Thus the isolation and characterisation of the above related lactonic constituents of *P. pabularia* provide a unique example of structure elucidation of minor components if a group of biogenetically related natural products from a single plant source be available.

## EXPERIMENTAL

The m.ps were determined in a Kofler block and are uncorrected. The UV absorption spectra were measured with a Beckman DK-2 Spectrophotometer and a Carl Zeiss Universal Spectrophotometer using 95% aldehyde-free EtOH and the IR spectra with a Perkin-Elmer Infracord Spectrophotometer in Nujol mull. The NMR spectra were recorded with Varian A 60 D and Varian HA-100 instruments with TMS as the internal standard. Preparative TLC was carried out with silica gel G as adsorbent, the developing system being benzene: EtOAc (4:1). The spots were detected with iodine vapour. The analytical samples were routinely dried *in vacuo* at 80° over  $P_2O_5$  for 24 hr.

### Isolation of the lactonic constituents

The air dried and milled roots of *P. pabularia* (1.6 kg) were exhaustively extracted with petrol (b.p. 60–80°) in a Soxhlet apparatus (75 hr). A semi-solid mass separated out in the flask. After decanting off the soln the residual pale yellow gum was thoroughly churned with chilled ether, filtered and crystallized from EtOAc. The granular solid (19.7 g), m.p. 138°, was identified as oxypeucedanin,  $C_{16}H_{14}O_5$  ( $M^+$  286) from m.m.p., co-TLC behaviour and comparison of spectral data with that of an authentic sample.

The concentrated soln of the crude extract was then chromatographed over silica gel (800 g), the column being eluted with solvents of increasing polarity using petrol, benzene, chloroform and methanol in different proportions. The light petrol, petrol:benzene (3:1) and petrol:benzene (1:1) fractions afforded osthol (14 g),  $C_{13}H_{16}O_3$  ( $M^+$  244), m.p. 83–84°. Imperatorin (2 g),  $C_{16}H_{14}O_4$  ( $M^+$  270), m.p. 99–100° migrated out with petrol:benzene (1:1), isooxypeucedanin (500 mg),  $C_{16}H_{14}O_5$  ( $M^+$  286), m.p. 142–144° with petrol:benzene (1:3) as eluents. The benzene eluate afforded pabularinone (100 mg), and later alloimperatorin (18 mg),  $C_{16}H_{14}O_4$  ( $M^+$  270), m.p. 212° (dec.). Pabulenol (80 mg) was obtained with benzene:chloroform (1:3) and chloroform as eluents.

Pabularinone,  $C_{16}H_{14}O_5$  ( $M^+$  286) was purified by repeated crystallizations from benzene:petrol (3:1)

mixture from which it was obtained as glistening prisms, m.p. 131–132°,  $[\alpha]_D^{25} = 0^\circ$  (Found: C, 66.91; H, 4.78; O, 28.23.  $C_{16}H_{14}O_5$ , requires: C, 67.13; H, 4.93; O, 27.9%).

#### *Acid catalysed cleavage of pabularinone to xanthotoxol*

Pabularinone (70 mg) was refluxed with glacial AcOH (2 ml) and conc  $H_2SO_4$  (2 drops) in an oil bath for 1 hr at 120–125°. The cooled soln was then poured over crushed ice-chips. The yellow ppt was filtered. It crystallized from acetone (yield: 40 mg) m.p. 248° (Found: C, 65.21; H, 2.86; O, 31.82.  $C_{11}H_6O_4$ , requires: C, 65.35; H, 2.99; O, 31.66%).

#### *Methylation of xanthotoxol*

Xanthotoxol (40 mg) was treated with excess diazomethane in methanolic soln and left for 7 days in an ice bath. On removing the excess diazomethane the reaction product was found to be a mixture of two components which were separated by preparative TLC. The product (7 mg) with  $R_f$ : 0.35 crystallized from acetone as colourless needles, m.p. 147–148°, and gave undepressed mixture m.p. and superimposable IR spectra with authentic xanthotoxin (Found: C, 66.52; H, 3.62; O, 29.72.  $C_{12}H_8O_4$ , requires: C, 66.67; H, 3.73; O, 29.60%).

#### *Oximation of pabularinone*

Hydroxylamine hydrochloride (100 mg) and anhyd NaOAc (1 g) were boiled with abs alcohol (20 ml) for 20 min. The soln was slightly cooled and the precipitated NaCl was removed by filtration. Pabularinone (20 mg) was dissolved in the clear filtrate and the resulting yellow soln was refluxed for 2 hr. A portion of the alcohol was then distilled off. The white crystalline ppt was dissolved in water (50 ml) and the residual alcohol was removed. The turbid aqueous soln was extracted with ether. The grey-white solid which crystallized out of the ether concentrate, m.p. 195° (dec), on preparative TLC furnished xanthotoxol (10 mg),  $C_{11}H_6O_4$  ( $M^+ 202$ ), m.p. 248°,  $R_f$ : 0.20 (Found: C, 65.20; H, 2.86; O, 31.85.  $C_{11}H_6O_4$ , requires: C, 65.35; H, 2.99; O, 31.66%) and pabularinone oxime (7 mg),  $C_{16}H_{15}NO_5$  ( $M^+ 301$ ) m.p. 142–144°,  $R_f$ : 0.35 (Found: C, 63.65; H, 4.82; O, 26.76; N, 4.62.  $C_{16}H_{15}NO_5$ , requires: C, 63.79; H, 4.98; O, 26.58; N, 4.65%).

#### *Synthesis of Pabularinone*

(a) *Epoxidation of imperatorin*. Imperatorin (1.3 g) was dissolved in dry chloroform and treated with perbenzoic acid soln in the same solvent (100 ml: 1.2g) and kept at room temp for 75 hr. The mixture was diluted with ether and washed with sat  $NaHCO_3$  aq. The solvent was removed and the red gum was dissolved in benzene and chromatographed over silica gel (18 g). The oily substance (700 mg) obtained with benzene as eluent crystallized from benzene:petrol, m.p. 112–114°,  $R_f$ : 0.39 (Found: C, 66.85; H, 4.81; O, 28.22.  $C_{16}H_{14}O_5$ , requires: C, 67.13; H, 4.93; O, 27.94%).

(b) *Treatment of oxyimperatorin with  $BF_3 \cdot etherate$* . Oxyimperatorin (100 mg) was dissolved in dioxan (25 ml) and  $BF_3 \cdot etherate$  (5 ml) was added. Instantly the soln became warm. It was cooled to room temp. After 30 min the deep red mixture was diluted with chloroform (50 ml), washed successively with water,  $NaHCO_3$  aq and again with water. The chloroform concentrate on preparative TLC afforded pabularinone (50 mg) which gave undepressed mixture m.p., identical  $R_f$  value: 0.40 and superimposable IR spectra.

*Pabulenol* was isolated from benzene:chloroform (1:3) and chloroform as eluents enveloped in a deep red jelly like covering. This dissolved in warm ethanol (40–45°) revealing the presence of a pale yellow solid. This was purified by repeated crystallization from benzene:petrol, m.p. 134–135°,  $[\alpha]_D^{25} = -3.8^\circ$  (EtOH),  $R_f$ : 0.78 [developing system—ethanol:EtOAc (1:3)]. (Found: C, 66.70; H, 4.82; O, 28.28.  $C_{16}H_{14}O_5$ , requires: C, 67.13; H, 4.93; O, 27.94%).

#### *Acetylation of pabulenol*

Pabulenol (20 mg) was dissolved in pyridine (5 ml) and treated with  $Ac_2O$  (1 ml) and left at room temp for 22 hr. The mixture was poured over crushed ice-chips. An oil separated out which solidified after vigorous shaking. This was taken up in chloroform (50 ml). The organic layer was made pyridine free. The solid obtained on removal of chloroform crystallized from hexane as shining needles (15 mg), m.p. 119–119.5°,  $R_f$ : 0.51 (Found: C, 65.70; H, 4.74; O, 29.38.  $C_{18}H_{16}O_6$ , requires: C, 65.85; H, 4.91; O, 29.24%).

#### *Rearrangement of pabulenol to isooxypeucedanin*

Pabulenol (20 mg) was refluxed with 10%  $H_2SO_4$  (9 ml). After the compound dissolved (1 hr) the soln was refluxed for a further period of  $1\frac{1}{2}$  hr. The cooled soln was shaken with chloroform (50 ml). The organic

layer on preparative TLC afforded isooxypeucedanin (10 mg), m.p. 140–142°,  $R_f$ : 0.50 and oxypeucedanin hydrate (7 mg), m.p. 129–130°,  $R_f$ : 0.70 [developing system—EtOH: EtOAc (1:3)]. Both these compounds were identified from co-TLC behaviour and superimposable IR spectra with authentic samples.

#### *Hydrogenation of pabulenol*

Pabulenol (15 mg) was dissolved in 95% aldehyde-free EtOH (10 ml) and hydrogenated for 45 min in the presence of 10% Pd/C (20 mg) as catalyst. Preparative TLC of the mixture (developing system—CHCl<sub>3</sub>: MeOH (95:5)) afforded dihydrobergaptol,  $R_f$ : 0.43 and tetrahydropabulenol,  $R_f$ : 0.58. These compounds were characterized from their mass fragmentation pattern.

#### *Synthesis of pabulenol*

Oxypeucedanin (100 mg) was dissolved in dioxan (20 ml) and BF<sub>3</sub> etherate (1 ml) was added at room temp. After 30 min the mixture was worked up. Preparative TLC of the chloroform concentrate afforded an appreciable amount of isooxypeucedanin (60 mg) C<sub>16</sub>H<sub>14</sub>O<sub>5</sub> (M<sup>+</sup> 286) m.p. 140–142°, and a solid m.p. 120–122°. Spectral analysis showed the latter to be a mixture of pabulenol (M<sup>+</sup> 286) and another component (M<sup>+</sup> 306). However, the two constituents could not be separated on a TLC using various solvent systems. Separation by column chromatography and even by acetylation proved futile. This mixture was finally resolved on a LKB MS gas liquid chromatograph. 2 μl of the chloroform soln of this mixture was injected into a silicone SE30 column, the temp being maintained at 220°. Helium was used as the carrier gas. An identical experiment was carried out with the pure natural product. The minor constituent in the mixture showed a retention time of 5 min. The major component and the natural product exhibited the same retention time of 5 min–44 sec and identical mass spectra thereby confirming the identity of these two compounds.

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