*Tetrahedron* Vol 24. pp 497 to 510. Pergamon Press 1968. Printed in Great Britain

# NATURAL CHROMENES—III*

## COLOURING MATTERS OF WARS: THE STRUCTURE OF FLEMINGINS A, B, C AND HOMOFLEMINGIN†

G. CARDILLO, L. MERLINI and R. MONDELLI

Politecnico, Istituto di Chimica,‡ Milano, Italy

(*Received in UK* 20 *March* 1967; *accepted for publication* 23 *May* 1967)

**Abstract**—Four new chalcones, flemingins A, B and C (I, II, III) and homoflemingin (IV) have been isolated from the dichloroethylene extract of the drug Wars, obtained from the seeds of *Flemingia rhodocarpa* Baker. I, II and III contain a 2-methyl-2(4'-methylpent-3'-enyl) chromene ring, whereas IV has a geranyl side chain. Their structures, which show an unusual oxygenation pattern, were established on the basis of chemical and spectrochemical evidence.

WARS (or Wurrus, or black kamala) is a drug prepared in East Africa by scraping the seed pods of *Flemingia rhodocarpa* Baker (Leguminosae) and usually sold on the market of Aden.[1] It appears as a brown-red powder, and it is used as cosmetic, dye and as adulterant of the more usual kamala. A very similar drug, from other *Flemingia* species, is known in India.[1]

Indian Wars was first investigated by Perkin,[2] who extracted a red resin, and fractionated it by crystallization into two products which he named flemingin and homoflemingin. In an attempt to degrade flemingin by alkali fusion, he obtained salicylic and acetic acids. The subject was not reexamined until 1957, when Pavolini and Gambarin[1] established definitely the botanical origin of the drug. Their experiments, however, threw no further light on the chemical constitution of the pigments.

We wish to report here the results of our investigation of the dichloroethylene extract of the drug. Careful chromatography of the extract through silica gel afforded four main orange-red substances, *flemingin A* (I), $C_{25}H_{26}O_5$, *flemingin B* (II) and *C* (III), both $C_{25}H_{26}O_6$, and *homoflemingin* (IV) $C_{26}H_{30}O_6$. The analytical data, indicating oxygenated aromatic structures, and the absorption spectra, suggest that they are flavonoids. Colour tests, i.e. deep red or violet colour with NaOH, red with conc. $H_2SO_4$, negative Shinoda reaction, and positive reaction[3] with $SbCl_5$, restricted the assignment to chalcone or aurone classes.

Flemingin C (III) melts at 180–182°, has $\alpha_D^{20} = +2{\cdot}3°$, and UV absorption maxima at 293 and 412 nm (ε 17,100, 17,200). Positive Dimroth[4] test, the bathochromic shift of 40 nm in the UV spectrum with $AlCl_3$,[5] and a C=O band at 6·15 μ in the IR indicate an OH strongly chelated with an adjacent carbonyl group. Intense absorption around 3·0 μ reveals the presence of other hydroxyls. With acetic anhydride and

* G. Cardillo and L. Merlini, *Tetrahedron Letters* 2529 (1967). Part II.

† Presented in part: *IUPAC International Symposium on the Chemistry of Natural Products*. Stockholm (1966).

‡ Centro del C.N.R. per la Chimica delle Sostanze Organiche Naturali.

sodium acetate, on a water bath flemingin C afforded a triacetate (V) $C_{31}H_{32}O_9$, whereas at higher temperature a tetraacetate (VI) $C_{33}H_{34}O_{10}$ was obtained, which gives no more reaction with $FeCl_3$. The UV spectrum of VI ($\lambda_{max}$ 259, 290 nm, $\varepsilon$ 26,500, 18,200) confirms the chalcone skeleton for these compounds.[5]

The NMR spectrum of III (acetone-$d_6$, Fig. 1) shows one chelated OH (sharp, 13·63 $\delta$), 3 phenolic OH (7·45, 7·91, 8·65 $\delta$), and an AB quartet with $J$ = 15·5 Hz (CH=CH *trans*); the strong downfield shift of these two protons (7·83 and 8·20 $\delta$) provides evidence for the chalcone sequence: Aryl—CH=CH—CO—Aryl. The aromatic part of the spectrum shows a singlet at 7·49 $\delta$, and an ABX pattern of five lines: a triplet for the X part ($\delta_X$ = 7·28) and a doublet for the AB ($\delta_A = \delta_B$ = 6·85),

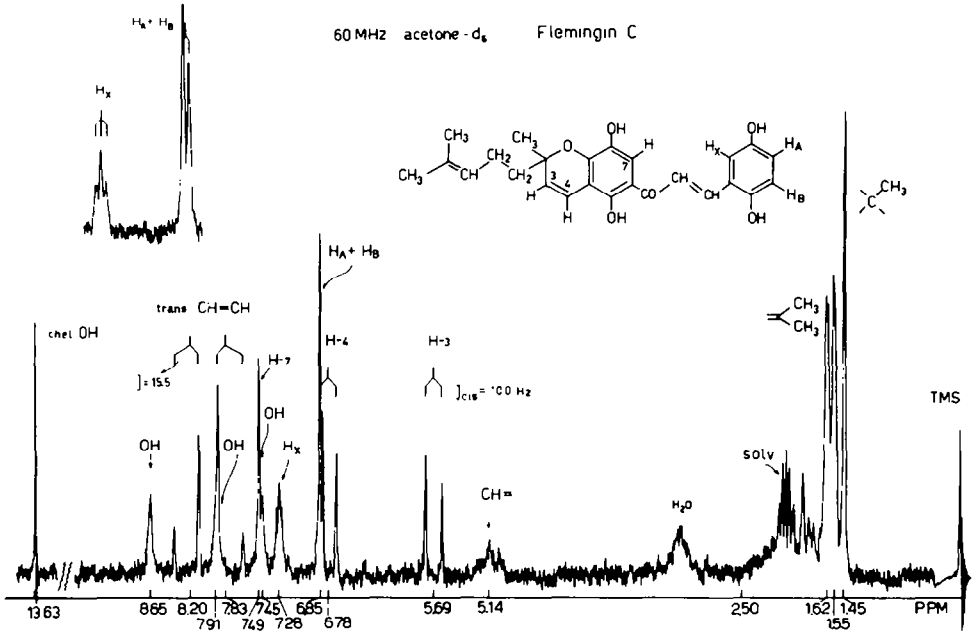
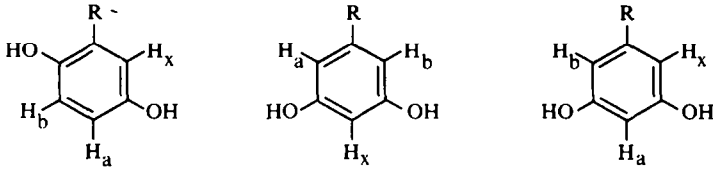



FIG. 1.

with the same separation of 1·5 Hz, partially overlapped by another signal. Because of the same chemical shift of $H_A$ and $H_B$, $J_{AB}$ can not be read, but this does not imply necessarily $J_{AX} = J_{BX}$ = 1·5, the separation of lines being equal to $\frac{1}{2}(J_{AX} + J_{BX})$.[6] By assuming $J_{para} \sim 0·5$, $J_{meta}$ = 1·5–2·5 and $J_{ortho}$ = 7·5–8·5 Hz, the spectrum is consistent with a 1,2,4 sequence of aromatic protons as shown in structure A.* That this is the correct assignment comes from the consideration that $H_A$ and $H_B$ have the same chemical shift, and they are at higher field (6·85 $\delta$) than $H_X$ (7·28 $\delta$).

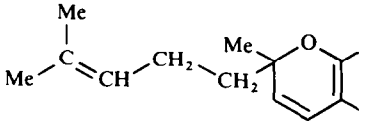



A B C

* The 1.2.3 sequence is immediately ruled out: $\frac{1}{2}(J_{meta} + J_{ortho})$ gives the value of 4·5–5·5 Hz.

The shielding effect experienced by $H_A$ and $H_B$ must be correlated with the presence of the same OH substituent in *ortho* position to each. A proton between two OH should lie at 6·0–6·3 $\delta$. The lower field shift of $H_X$ indicates absence of adjacent hydroxyls, or a position between one OH and one electron-withdrawing substituent. Consequently structures like B and C can be ruled out even if they fit with the coupling constants values.

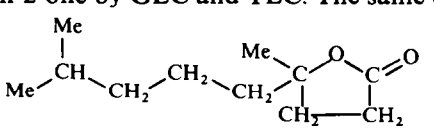
The singlet at 7·49 $\delta$ must come from a proton on another benzene ring, whereas the AB pattern of two doublets at 6·78 and 5·69 $\delta$ ($J$ = 10·0) is unambiguous evidence of the presence of a chromene ring.[7] The high-field absorption integrates for 13 protons,* among which three tertiary methyls are clearly seen at 1·45, 1·55 and 1·62 $\delta$. The two latter have a small splitting of 1 Hz, which must be correlated with the olefinic proton at 5·14 $\delta$, which appears roughly as a triplet ($J \sim 7$) complicated by other small interactions. Consequently they are easily arranged in an isoprenic chain $(CH_3)_2C{=}CH{-}CH_2{-}$, which, however, is not linked to an aromatic ring, because of the high field shift of the methylene ($\delta < 2{\cdot}5$). Taking into account that the remaining two protons occur around 1·5–2·5 $\delta$ and that only one Me (1·45 $\delta$) is still available, we can put forward the sequence D:



D

Flemingin C triacetate was hydrogenated with Pd in EtOH to hexahydroflemingin C triacetate (VII), $C_{31}H_{38}O_9$, $\lambda_{max}$ 286, 325 nm. The NMR spectrum of VII ($CDCl_3$) indicated that saturation of the three double bonds had occurred. The symmetrical absorption of 4H centered at 3·02 $\delta$ is consistent with the AA'BB' pattern**of the sequence —CO—$CH_2$—$CH_2$—Aryl, and the two rough triplets at 2·70 and 1·81 $\delta$ (splitting = 7 Hz) with a deceptively simple ABXY type spectrum[6] ($\delta_A \sim \delta_B$ and $\delta_X \sim \delta_Y$) due to the methylenes of the chromane ring. The saturated side-chain gives the doublet of the isopropyl group at 0·88 (6 H), and a complex absorption of 10 H between 1·0 and 1·8 $\delta$ (comprising the Me—C(—)—O— singlet that is still at 1·43 $\delta$), whereas the aromatic part of the spectrum shows only four protons. Moreover, three OAc groups at 2·26, 2·28, 2·30 and a chelated OH at 12·90 $\delta$ were observed.

Modified[8] Kuhn–Roth oxidation of VII gave a volatile ketone, which was identified as 6-methylheptan-2-one by GLC and TLC. The same chromic oxidation under



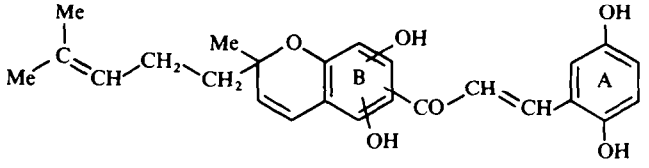
VIII

* The exact value of this integral was obtained from the spectrum of the triacetate, soluble in $CDCl_3$, in order to exclude the absorption of acetone isotopic impurity.

** The symbols adopted are those used by J. W. Emsley, J. Feeney and L. H. Sutcliffe, *High Resolution Nuclear Magnetic Resonance Spectroscopy*. Pergamon Press, Oxford (1965).

conditions given by Isler *et al.*[9] gave 4,8-dimethyl-4-hydroxynonanoic acid lactone (VIII). This result is consistent with the presence in flemingin C of a 2-methyl-2-(4'-methylpent-3'-enyl)chromene ring (sequence D) with an asymmetric carbon atom, as indicated by the optical activity of flemingin C.

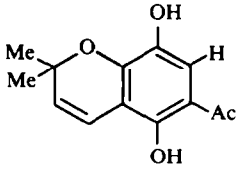
Ozonolysis of V or VI led to the isolation of a carbonyl compound, $\lambda_{max}$ 246, 295 nm, IR 5·68 (OAc) and 5·90 (CHO) μ. The NMR spectrum ($CCl_4$) shows 1 CHO (s, 10·10 $\delta$), two acetyls (2·19 and 2·25 $\delta$) and three aromatic protons: ABC pattern with $\delta_A = 7{\cdot}12$, $\delta_B = 7{\cdot}28$, $\delta_C = 7{\cdot}55$ (*ortho* to CHO), $J_{AB} = 8{\cdot}5$ (*ortho*), $J_{BC} = 2{\cdot}5$ (*meta*) and $J_{AC} = 0{\cdot}6$ Hz (*para*). The compound is thus 2,5-diacetoxybenzaldehyde, as confirmed by comparison with an authentic sample, by TLC, UV and IR spectra. The aromatic substitution corresponds to the three protons system present in flemingin C. Because this fragment must come from ring A of the chalcone skeleton, the chromene ring must be attached to the ring B, as shown in the partial formula E:



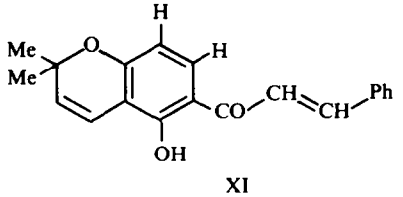

E

Similarly, ozonolysis and $KMnO_4$ oxidation of flemingin C tetramethyl-ether (IX) gave respectively 2,5-dimethoxybenzaldehyde and 2,5-dimethoxybenzoic acid. It was not possible to isolate any fragment from ring B. Alkali fusion yielded again only gentisic acid, which is probably the compound m.p. 182–186° obtained by Pavolini[1] under similar conditions.

Inspection of partial formula E shows that the two remaining hydroxyls must be attached to ring B. The presence of a chelated OH has been already pointed out. The NMR spectrum of III provides good evidence that only one OH is *ortho* to the carbonyl. Thus only one sharp signal appears at low field (13·63 $\delta$), whereas the presence of two *ortho* OH should give rise to a less sharp band at higher field, as in phloroacetophenone derivatives (10·4–11·7 $\delta$).[10] Moreover, the singlet due to the aromatic proton on ring B appears at 7·49 $\delta$, which is a strong evidence that it experiences the deshielding effect of the adjacent carbonyl group, together with the shielding effect of one OH in *ortho* position, and excludes that it lies between two oxygens.* This result rules out the usual phloroglucinol substitution for ring B.



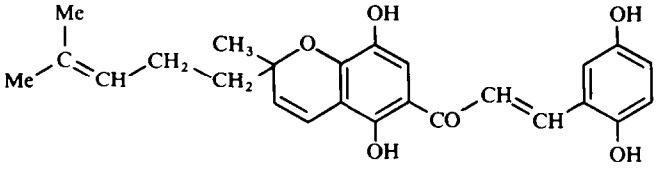

X




XI

* Compare the shift of the aromatic hydrogen in X (7·21$\delta$, acetone-$d_6$. Moreover, in lonchocarpin (XI) the proton *peri* to the chromene oxygen absorbs at 6·41 $\delta$ (acetone). The presence of another OH in *ortho* position to this proton (phloroglucinol substitution) should shift it further upfield (ca. 6·0 $\delta$).

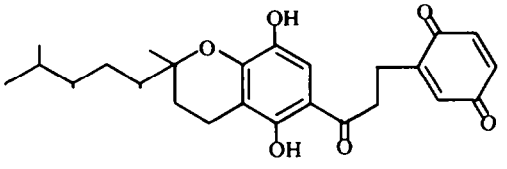
At this point it was observed that acetylation of the fourth OH in flemingin C triacetate produced a shift (in $CDCl_3$) resp. of $\Delta\delta = +0{\cdot}36$ on proton 4 and of $\Delta\delta = -0{\cdot}16$ on proton 3 of the CH=CH group in the chromene ring. Similar results in a series of synthetic models and other natural 5-hydroxychromenes have shown that this effect appears *only* when the OH *peri* to the chromene double bond is acetylated.[11] Particularly, because flemingin C triacetate shows still a chelated OH signal at 13·50 $\delta$, and acetylation of this OH produces the described effect on the chromene protons, it is reasonable that the chelated OH is peri to the chromene double bond. Together with the above discussed evidence, this establishes consequently the whole substitution on ring B, and thus the structure III for flemingin C:



III

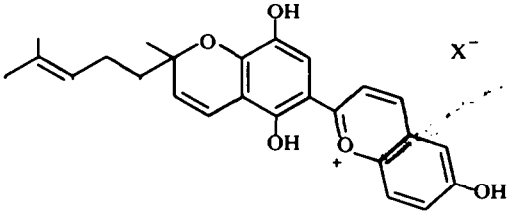
This compound presents an unusual oxygenation pattern in both rings A and B; furthermore, only two examples of natural products with an isoprenic $C_{10}$ chain closed to form a chromene ring are so far known: cannabichromene[12] and gambogic acid.[13]

Attempts to oxidize flemingin C to the corresponding bis-quinone with $FeCl_3$ or other oxidants failed. Treatment of hexahydroflemingin C with $K_2Cr_2O_7$ in acetic acid gave instead a red insoluble product XII, where, however, oxidation had occurred only in ring A, as appears from the mass spectrum ($M^+ = m/e$ 426), from the NMR spectrum, which still shows a chelated OH, and from the appearance of a new C=O band at 6·05 $\mu$ in the IR.



XII

Attempts to cyclize III to the corresponding flavanone in HCl or acetic acid failed with formation of violet compounds, most probably the pyrilium salts.



Flemingin A (I), m.p. 148–150°, $[\alpha]_D^{20} = -4{\cdot}2°$, $C_{25}H_{26}O_5$, has an UV absorption very similar to that of flemingin C, but with a hypsochromic shift of the longer wavelength maximum ($\lambda_{max}$ 295, 376 nm). Mild acetylation gave a diacetate XIII with a chelated OH not acetylated; a triacetate was obtained under stronger conditions.

The NMR spectrum (acetone-$d_6$, 100 MHz) of flemingin A (Fig. 2) shows patterns very similar to those of flemingin C, except for the presence of only three OH, and for

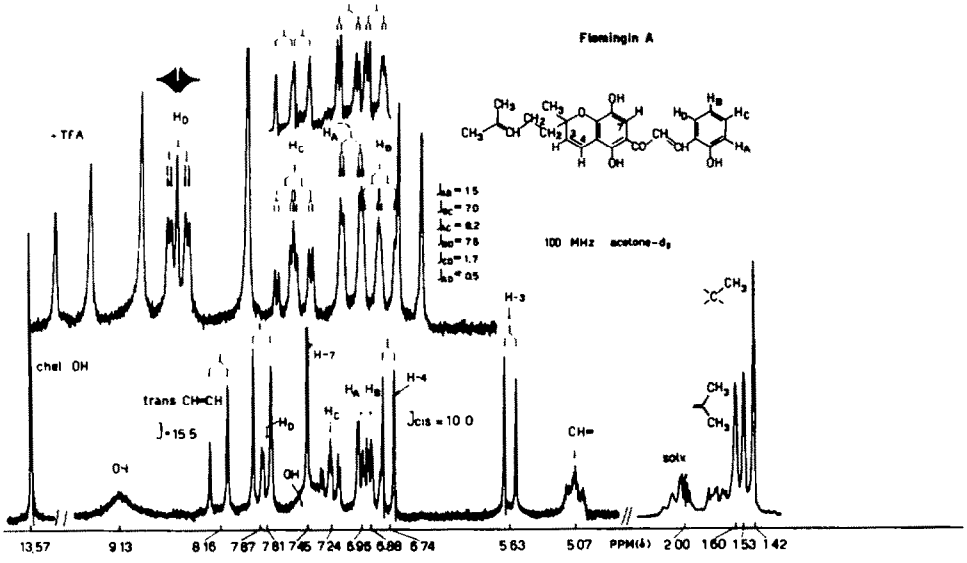
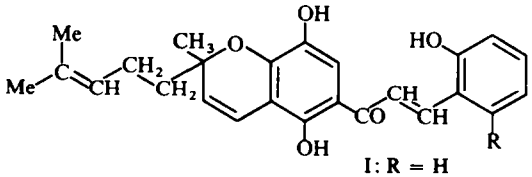



FIG. 2.

the aromatic hydrogens of ring A. The 1st order analysis of this four spin system gives: $J_{AB} = 1{\cdot}5$, $J_{AC} = 8{\cdot}2$, $J_{BC} = 7{\cdot}0$, $J_{BD} = 7{\cdot}5$, $J_{CD} = 1{\cdot}7$, $J_{AD} \leqslant 0{\cdot}5$ Hz; $\delta_A = 6{\cdot}96$, $\delta_B = 6{\cdot}88$, $\delta_C = 7{\cdot}24$, $\delta_D = 7{\cdot}81$. These data were confirmed by spin-decoupling experiments. With irradiation of $H_D$, $J_{BD}$ and $J_{CD}$ vanish, and as a consequence the double triplet of $H_B$ collapses into a double doublet with splittings of 7·0 ($J_{BC}$) and 1·5 Hz ($J_{AB}$), the signal of $H_C$ loses the small splitting of 1·7 Hz and changes into two doublets of 7·0 ($J_{BC}$) and 8·2 ($J_{AC}$) Hz.* It follows that the four protons of ring A are all *ortho* to each other: also their chemical shifts are consistent with the presence of only one OH on ring A. As expected, ozonolysis of flemingin A diacetate gave *ortho-acetoxybenzaldehyde*, identified by comparison with an authentic sample.

On conventional flemingin A diacetate to triacetate a shift ($\Delta\delta = +0{\cdot}35$ for proton 4 and $\Delta\delta = -0{\cdot}13$ for proton 3) of the chromene ring protons was observed in the NMR spectrum, as in the corresponding derivatives of flemingin C, and the singlet of the aromatic hydrogen on ring B lies at the same value as in flemingin C. It is reasonable to conclude that both compounds have the same substitution on ring B. Flemingin A has thus the following structure (I):

* The small *para* interaction between $H_A$ and $H_D$($J_{AD} \leqslant 0{\cdot}5$ Hz) becomes visible only by double irradiation, with the sharpening of the four lines of $H_A$ signal.



I: R = H
II: R = OH

Flemingin B (II) appears to be isomer of flemingin C, $C_{25}H_{26}O_6$, and has m.p. 176–178°, $[\alpha]_D^{20} = +6{\cdot}7°$, UV maxima at 278, 285, 376 nm. It is similar to flemingin C with respect to colour tests, acetylation and methylation. The NMR spectrum (acetone-$d_6$, Fig. 3) shows the same high-field pattern and olefinic absorptions (chalcone sequence, chromene ring, and side-chain) as flemingins A and C. The four phenolic hydroxyls lie at 7·44, 9·15 (2 H) and 13·73 $\delta$ (chelated). The aromatic hydrogen on ring B appears at 7·34 $\delta$. The three aromatic protons on ring A appear as an $AB_2$ pattern, with $J_{AB} = 8{\cdot}2$ Hz, $\delta_A = 6{\cdot}95$ and $\delta_B = 6{\cdot}51$. The chemical shift of the two equivalent $H_B$ indicates the presence of one OH in *ortho* or *para* position, and the one substitution on ring A consistent with these data is that shown in Fig. 3. Again

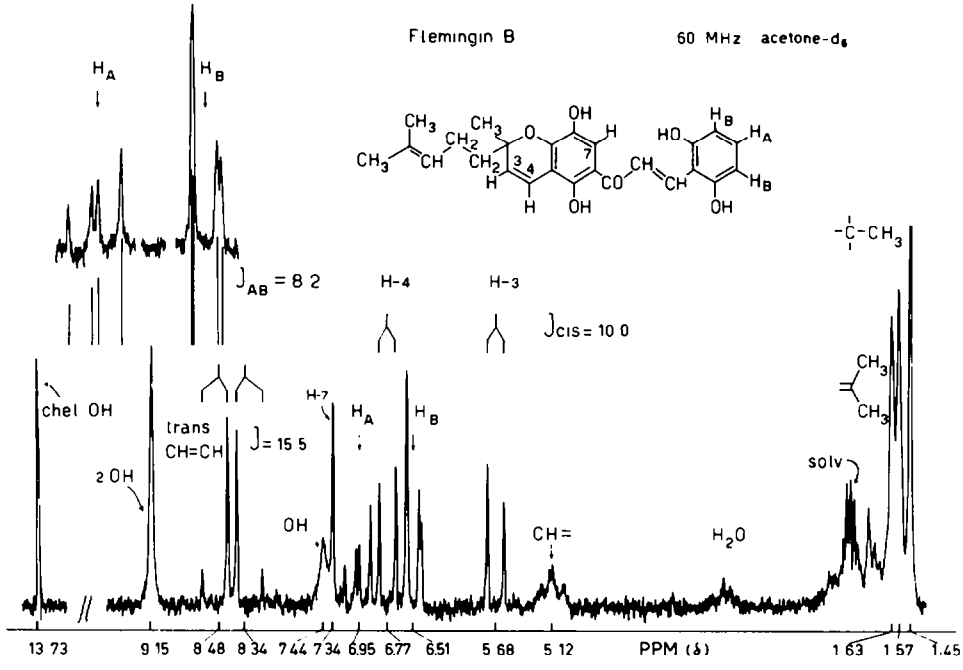



FIG. 3.

ozonolysis of the triacetate XIV confirmed this conclusion, giving 2,6-diacetoxybenzaldehyde, identified by UV, IR and NMR spectra. The same arguments employed before for I can be repeated now for flemingin B, to which structure II has been assigned.

The fourth pigment, homoflemingin (IV), $C_{26}H_{30}O_6$, m.p. 160–163°, optically inactive, shows UV absorption at 272, 317 and 404 nm, and IR prominent bands at 3·0 (OH) and 6·15 μ (chelated CO). Its NMR spectrum (acetone-$d_6$, Fig. 4) shows a

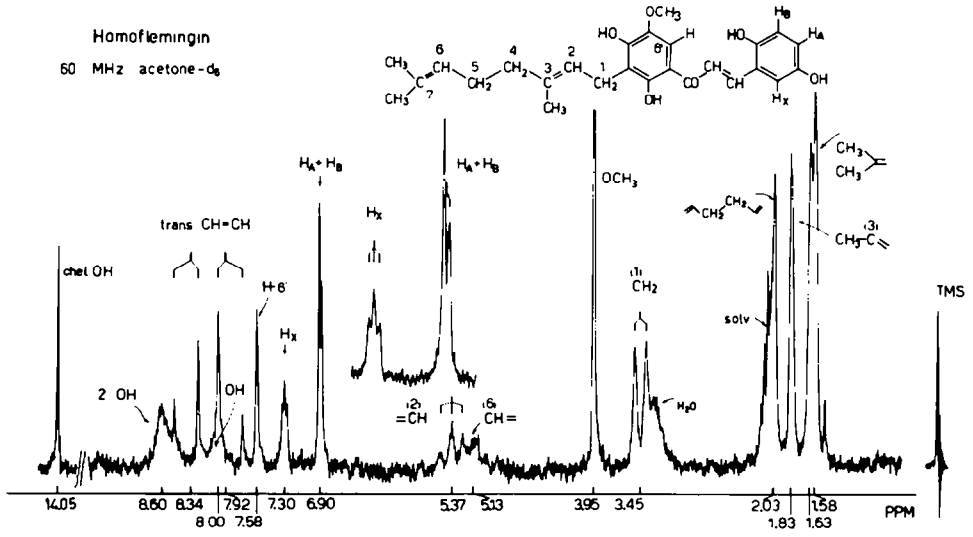
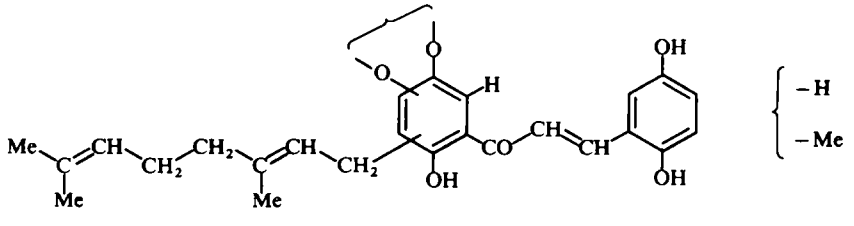


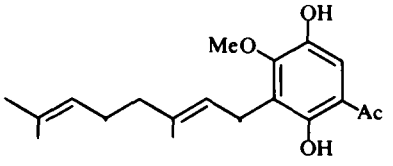

FIG. 4.

chelated OH (14·05 $\delta$), a CH=CH *trans* (chalcone), and the aromatic substitution identical to that in flemingin C, i.e. the deceptively simple ABX pattern of five lines for ring A, and the singlet of the proton on ring B at 7·58 $\delta$. It lacks however the chromene signals, and shows instead two almost superimposed vinylic protons, which appear roughly as two triplets ($J \sim 7$) complicated by allylic couplings, a doublet of 7 Hz (2H) at 3·45 $\delta$ (Aryl-$CH_2$—CH=), an isopropylidene group $Me_2C$=, 1·58 and 1·63 $\delta$), and one Me on a tertiary unsaturated carbon (doublet, $J_{all} \sim 1$ Hz; 1·83 $\delta$), plus four other nearly equivalent protons centered at 2·03 $\delta$ ($\gtrless$—$CH_2$—$CH_2$—$\gtrless$). All these seventeen protons can best be arranged in a geranyl side chain. In addition, a OMe group appears at 3·95 $\delta$, plus three phenolic OH. Homoflemingin gave a triacetate (XV) $C_{32}H_{36}O_9$, $\lambda_{max}$ 312, 395 nm, which contains still a chelated OH, as shown by its low-field absorption in the NMR spectrum. Ozonolysis of XV afforded 2,5-diacetoxybenzaldehyde. This establishes the nature of the ring A of the chalcone, and, together with the NMR data, indicates the following partial structure F for homoflemingin:

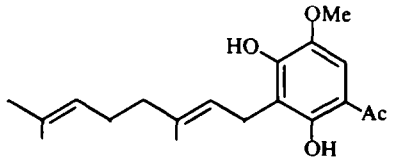



F

Alkaline degradation with $Ba(OH)_2$ of homoflemingin gave a ketone (G) $C_{19}H_{26}O_4$. From the NMR spectrum it appears that this compound still holds the side chain of homoflemingin; the aromatic absorption due to the ring A and the chalcone group having disappeared, and a new singlet (2·48 $\delta$, $COCH_3$) being present. The

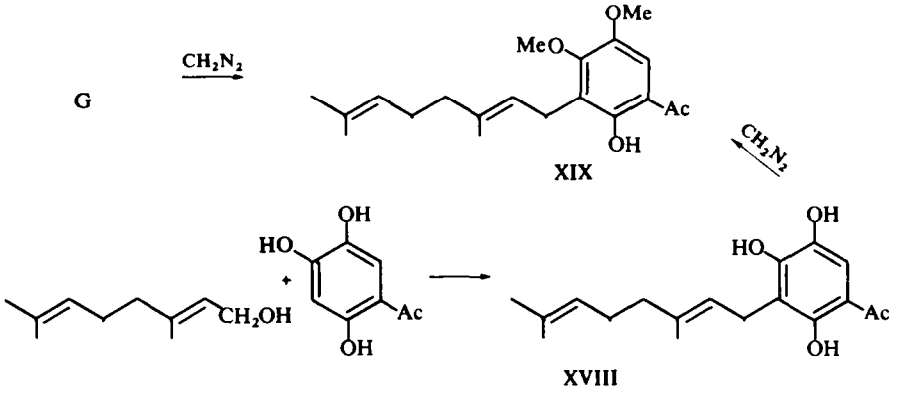


XVI



XVII

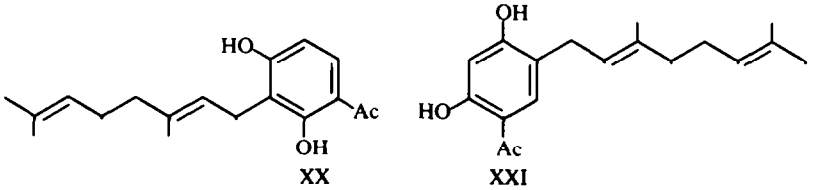
four possible formulae for the ketone could be reduced to XVI and XVII by the synthesis of the methylated derivative XIX, by reaction of geraniol with 2,4,5-trihydroxyacetophenone in decalin,[12a] and methylation of the product XVIII with diazomethane. The compound XIX was identical with that obtained by methylation of the ketone G with diazomethane, as shown by comparison of UV, IR and NMR spectra and TLC behaviour.



XIX

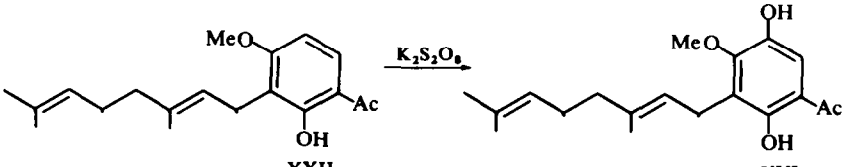
XVIII

The unambiguous distinction between XVI and XVII became possible only with the synthesis of both compounds. Alkylation of 2,4-dihydroxyacetophenone lithium salt with geranyl bromide in benzene[14] gave only the C-alkylated products XX and XXI, separated by chromatography and easily identified by their NMR spectra.



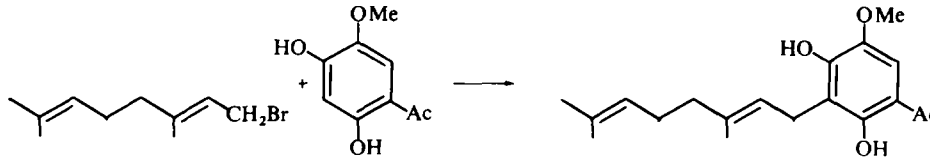
XX XXI

Methylation of XX with one mole of $Me_2SO_4$ afforded XXII, the Elbs persulfate oxidation of which gave the desired product XVI.



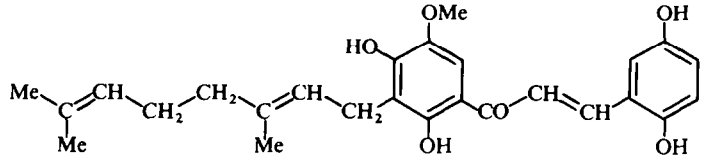
XXII XVI

Compound XVII was obtained by alkylation of 2,4-dihydroxy-5-methoxy-acetophenone (XXIII) lithium salt with geranyl bromide in benzene:



XXIII XVII

The m.p. and the spectral data are identical with those of the degradation product (G) of homoflemingin; the formula of this latter is thus established as IV:



IV

## EXPERIMENTAL

NMR spectra were measured with A-60 or HA-100 Varian spectrometers. Spin decoupling experiments were performed with the "frequency sweep" method. The integrals were measured with a 405/CR Hewlett-Packard digital voltmeter. Chemical shifts are in ppm ($\delta$) from TMS, used as the internal standard, coupling constants are in Hz. In the text, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. IR spectra (values in μ) of Nujol mulls were recorded with a Perkin–Elmer Infracord, UV spectra (values in nm) of solutions in 95% EtOH with a Beckman-DK-2 apparatus. Mass spectra were recorded with a Hitachi RMU6D spectrometer (single focus, 70 eV, 80 μA, direct inlet system, 250°). Silica gel Merck 0·05–0·20 mm was used for column chromatographies, for TLC it was Merck G.

*Extraction.* Good quality commercial Wars (50 g) purchased in Asmara, Ethiopia, was extracted with hexane in a Soxhlet extractor for 4 days to remove wax. The residue was extracted with 1,2-dichloroethylene until the extract was no longer coloured (at least 5 days). Concentration of the extract gave a red resin (18 g), which solidified (4 g) after treatment with cold $CHCl_3$ or dichloroethylene. Chromatography of the crude solid product (3·5 g) through silica gel (300 g) with hexane–AcOEt (4:1) gave in the order: flemingin A (32 mg), mixture of flemingin A and B, flemingin B (550 mg), mixture of flemingin B and C, flemingin C (315 mg), mixture of flemingin C and homoflemingin, homoflemingin (230 mg). Final elution with AcOEt gave only resinous products. Flemingin A and homoflemingin were contaminated each with a minor component; these have not yet been studied, due to the small amount. Mixtures of flemingin B and C could be easily separated by chromatography through silica gel with $CHCl_3$–ether (2:1); mixtures of flemingin C and homoflemingin by re-chromatography with hexane–AcOEt (4:1). Control of the purity of the fractions was carried on with TLC with hexane–AcOEt (1:1) or $CHCl_3$–ether (2:1).

*Flemingin A* (I) m.p. 148–150° ($CHCl_3$). (Found: C, 73·24; H, 6·72. $C_{25}H_{26}O_5$ requires: C, 73·86; H, 6·45%; UV: 295, 376 (ε 19,100, 23,200); $[\alpha]_D^{20} = -4·2°$ ($CHCl_3$, $c = 0·99$).

*Flemingin A diacetate* (XIII). Flemingin A (100 mg) in 3 ml $Ac_2O$ and 50 mg NaOAc were heated 5 min on steam bath, and left overnight. After evapn., taking up with water and extn. with ether, the extract was chromatographed with hexane–AcOEt (4:1) to give XIII, m.p. 145–147° from EtOH. (Found: C, 71·22; H, 6·21. $C_{29}H_{30}O_7$ requires: C, 71·00; H, 6·16%); UV: 230, 297–304, 358 (ε 17,600, 16,400, 18,900), $[\alpha]_D^{20} = -1·49°$ ($CHCl_3$ $c = 1$), NMR($CDCl_3$): 2 $=C-CH_3$ (1·60, 1·67), 1 $-O-\overset{|}{C}-Me$ (1·43), 4 H (1·0–2·5), 2 MeCO— (2·30, 2·38), 1 CH = (m, 5·1), 1 CH = CH (chromene ring, 5·56, 6·77, $J = 10$), 5 aromatics plus CH=CH (7·0–8·1), chelated OH (13·35).

*Flemingin A triacetate.* 100 mg of XII were refluxed 1 hr with $Ac_2O$ (3 ml) and AcONa (50 mg). Working up gave XIII as an oil, UV: 229, 295 (ε 29,900, 20,500). NMR ($CDCl_3$): 1 $Me-\overset{|}{C}-O$ (1·41), $Me_2C$ = (6H, 1·58), 4H (1·5–2·7), 3 MeCO— (2·29, 2·29, 2·33), 1 CH= (m, 5·1), 4 aromatic protons (7·0–7·9).

*Ozonolysis of* XIII. A soln. of 100 mg XII in 7 ml AcOEt was cooled with dry ice–acetone and a flow of ozonized $O_2$ was bubbled through for 40 min (the soln became colourless after 5 min). A few drops of water were added, the solvent evapd., the residue taken up with water and extd. with ether. Chromatography of the ext. with $CHCl_3$–ether (2:1) gave in the first fraction, *2-acetoxybenzaldehyde*, identified by TLC, GLC (Apiezon L, 163°, He) and IR comparison.

*Flemingin B* (II): m.p. 176–178° (toluene). (Found: C, 71·26; H, 6·21. $C_{25}H_{26}O_6$ requires: C, 71·07; H, 6·20%); UV: 278, 285, 376 (ε 13,900, 13,900, 27,600), $[\alpha]_D^{20} = +6·7°$ (EtOH, $c = 1·64$).

*Flemingin B triacetate* (XIV). Prepared as the corresponding derivative (XIII) of flemingin A melts at 115–119° from EtOH. (Found: C, 67·98; H, 5·85. $C_{31}H_{32}O_9$ requires: C, 67·87; H, 5·88%); UV: 229 sh, 293, 353 (ε 22,500, 24,000, 21,000). NMR ($CDCl_3$): 1MeC(—O—) (1·42), 2Me—C(=) (1·58, 1·67), 4H (1·3–2·4), 3MeCO— (2·28, 2·33, 2·33), 1CH(=) (m, 5·1), 1CH=CH (chromene ring, 5·57, 6·78, $J = 10$), 3 arom. H ($AB_2$: $\delta_A = 7·38$, $\delta_B = 7·06$), 1 arom. H (s, 7·34), 1 CH=CH (7·51, 7·70, $J = 15·5$), 1 chel. OH (13·26).

*Flemingin B tetraacetate.* Prepared as for flemingin A triacetate, and purified by chromatography with hexane–AcOEt (4:1) as an oil. (Found: C, 65·99; H, 5·73 . $C_{33}H_{34}O_{10}$ requires: C, 67·11; H, 5·80%); UV: 226, 283 (ε 27,600, 18,600). NMR ($CCl_4$): 1MeC(—O—) (1·39), 2Me—C(=) (1·57, 1·64), 4H (1·0–2·1), 4MeCO— (2·10–2·35), 1CH(=) (m, 5·05), 1CH=CH (chromene ring, 5·60, 6·35, $J = 10$), 1 arom. H (s, 7·01), 5H (6·8–7·4).

*Ozonolysis of flemingin B tetraacetate.* This was accomplished as described for XIII. The crude product was chromatographed with $CHCl_3$–ether (2:1) and the first fractions gave *2,6-diacetoxybenzaldehyde*, m.p. 92–93°[15] (benzene–ligroine); UV: 247, 295 (ε 10,900, 2240); IR: 5·65, 5·88. NMR ($CDCl_3$): 2MeCO— (s, 2·36), 3 arom. H ($AB_2$: $\delta_A = 7·56$, $\delta_B = 7·06$, $J_{AB} = 8·1$), 1CHO (s, 10·20).

*Hexahydroflemingin B.* Flemingin B (300 mg) in 100 ml dry MeOH were reduced with $H_2$ in the presence of 60 mg 5% Pd/C; 3 moles/mole were adsorbed. Evapn. and chromatography with hexane–AcOEt (1:1) gave 220 mg of white crystals, m.p. 145–146° from hexane–AcOEt. (Found: C, 70·10; H, 7·40. $C_{25}H_{32}O_6$ requires: C, 70·07; H, 7·53%); UV: 244, 289, 355 (ε 9200, 12,500, 8050). NMR ($CDCl_3$): $Me_2CH$— (0·85, d of 6 Hz), 7 H (1·0–2·0), 1 Me—C(—O—) (s, 1·30), —$CH_2$—$CH_2$— (chromane, decept. simple ABXY of two rough triplets, $\delta_A \sim \delta_B = 1·80$, $\delta_X \simeq \delta_Y = 2·64$) —CO—$CH_2$—$CH_2$—Aryl (AA'BB', center 3·16), 2OH (5·2), 3 arom. H ($AB_2$: $\delta_A = 6·93$, $\delta_B = 6·43$), 1 arom. H (s, 7·12), 2OH (6·91), 1 chel. OH (12·35).

*Flemingin C* (III) has m.p. 180–182° from toluene. (Found: C, 70·05; H, 6·32. $C_{25}H_{26}O_5$ requires: C, 71·07; H, 6·20%); UV: 293, 412 (ε 17,100, 17,200); $[\alpha]_D^{20} = +2·3°$ (EtOH, $c = 0·308$).

*Flemingin C triacetate* (V) was prepared as described for XIII, m.p. 159–161° from EtOH. (Found: C, 67·68; H, 5·84. $C_{31}H_{32}O_9$ requires: C, 67·87; H, 5·88%); UV: 230, 293, 356 (ε 22,900, 22,900, 23,000). NMR ($CDCl_3$): 1 Me—C(—O—) (1·42), 2 Me—C(=) (1·60, 1·67), CH=CH (chromene, 5·55, 6·78, $J = 10$), 3 arom. H (decept. simple ABX: $\delta_A = \delta_B = 7·15$, $\delta_X = 7·45$), 1 arom. H (s, 7·40), CH=CH (7·36, 7·84, $J = 15·5$), 1 chel. OH (13·50).

*Flemingin C tetraacetate* (VI) was prepared as described for flemingin A tetraacetate, m.p. 108–110° from benzene–hexane. (Found: C, 66·59; H, 5·92. $C_{33}H_{34}O_{10}$ requires: C, 67·11; H, 5·80; UV: 229, 290 (ε 26,500, 18,200); NMR ($CDCl_3$): very similar to the spectrum of V, except for the CH=CH (chromene, 5·71, 6·42, $J = 10$) and CH=CH (7·16, 7·64, $J = 15·5$).

*Hexahydroflemingin C.* Flemingin C (1·4 g) in 200 ml MeOH was hydrogenated with 86 mg $PtO_2$ as the catalyst. Evapn. and chromatography with hexane–AcOEt (4:1) gave 970 mg of the compound. m.p. 135°. (Found: C, 70·67; H, 7·73. $C_{25}H_{32}O_6$ requires: C, 70·07; H, 7·53%); UV: 243, 290, 352 (ε 8900, 14,200, 7500); NMR ($CDCl_3$): $Me_2CH$— (0·88, d of 6 Hz), 1 Me—C(—O—) (s, 1·32), 9 H (1·0–2·0), Aryl—$CH_2$—$CH_2$—CO— + Aryl—$CH_2$— (chromane) (6 H, 2·4–3·4), 3 arom. H (6·5–6·8), 1 arom. H (s, 7·05), 1 chel. OH (12·38), other OH (very broad, ca. 5·5).

*Hexahydroflemingin C triacetate* (VII). 178 mg of V in 40 ml MeOH were hydrogenated with 25 mg

$PtO_2$ as the catalyst. Evapn. and crystn. from EtOH gave VII (99 mg), m.p. 111–112°. (Found: C, 67·78; H, 7·04. $C_{31}H_{38}O_9$ requires: C, 67·13; H, 6·91%); UV: 286, 325 (ε 36,000, 17,100).

*Ozonolysis of* VI. This was carried on as described for flemingin A diacetate. A sample of the reaction mixture was treated with 2,4-dinitrophenylhydrazine. The phenylhydrazones were dissolved in ether and chromatographed through silica gel Mallinckrodt, 100 mesh, with hexane–ether (6:1). The first fractions contained the DNPH of acetone and acetaldehyde (this latter coming from the solvent). The following fractions contained the DNPH of 2,5-diacetoxybenzaldehyde, identified by comparions of IR spectrum, and TLC. The bulk reaction mixture, worked up as described before, was chromatographed with $CHCl_3$–ether (2:1), and gave 2,5-*Diacetoxybenzaldehyde* identified by IR, UV, NMR spectra and comparison with a synthetic sample; TLC: $R_f$ 0·48 (CHCl–ether, 2:1), 0·52 (benzene–ether–HCOOH, 50:50:1, yellow fluorescent in UV light).

*Kuhn-Roth oxidation of* VII. Hexahydroflemingin C triacetate (85 mg) were treated with 40 ml of the oxidizing soln prepared according to Karrer-Schmid,[8] and distilled. The distillate was neutralized and extd. with ether. It contained *2-methylheptanone*, identified by TLC and GLC (Carbowax 20M 10% on acid-washed Chromosorb, or Apiezon L on Chromosorb W-MMDS 60–80 mesh, column 5″ × ⅛″, carrier gas $N_2$).

*4,8-dimethyl-4-hydroxynonanoic acid lactone* (IX). Hexahydroflemingin C or B (970 mg) was oxidized with $CrO_3$–AcOH and treated as described by Isler *et al.*[9] 40 mg of IX, were obtained, identified by comparison with an authentic sample.

*Alkali fusion of flemingin C.* 100 mg of flemingin C were melted for 20 min with 2 g NaOH and a few drops of water. Acidification of the product, extn. with ether and chromatography with hexane–AcOEt (1:1) gave a compound which melted at 198° after sublimation, and was identified with *gentisic acid* by UV, NMR spectra and mixed m.p.

*Flemingin C tetramethylether* (IX). Flemingin C (1 g) was refluxed 15 hr with 5 ml $Me_2SO_4$, 50 ml dry acetone, 15 ml MeOH and 10 g $K_2CO_3$. Filtration, washing with acetone, evapn. and chromatography with benzene gave IX (600 mg), oil, NMR($CCl_4$): 1 Me—C(—)—O— (1·42), $Me_2C{=}CH$— (1·57, 1·63), —$CH_2$—$CH_2$— (1·7–2·3), 4 Me (3·67, 3·72, 3·83, 3·83), 1 CH═ (m, 5·06), CH═CH (chromene, 5·55, 6·61, $J$ = 10), 3 arom. H (decept. simple ABX: $\delta_A = \delta_B$ = 6·76, $\delta_X$ = 7·06), 1 arom. H (s, 7·14), CH═CH (7·56, 7·90, $J$ = 15·5).

*Ozonolysis of* IX. This was carried out as described before. Working up gave *2,5-dimethoxybenzaldehyde*, identified by NMR spectrum. $KMnO_4$ *oxidation of* IX. 500 g IX in 50 ml acetone were treated with 2·5 g $KMnO_4$ for 12 hr at room temp. Working up and chromatography with ether–benzene (1:1) gave *2,5-dimethoxybenzoic acid*, m.p. 72–73°.

*Hexahydroflemingin C tetramethyleter.* 680 mg of IX in 50 ml MeOH were hydrogenated with 50 mg $PtO_2$ as the catalyst. Evapn. and crystn. from EtOH gave 210 mg of the product, m.p. 85°. (Found: C, 72·02; H, 8·07; $C_{29}H_{40}O_6$ requires: C, 71·87; H, 8·32%); UV: 232sh, 287, 320sh (ε 22,200, 14,500, 7150); IR: 6·05. NMR($CCl_4$): $Me_2CH$— (0·91, d of 6 Hz), 1Me—C(—)—O— (1·29), 7H (1·0–1·7), —$CH_2$—$CH_2$— (chromane, decept. simple ABXY of two rough triplets, $\delta_A \simeq \delta_B$ = 1·74, $\delta_X \simeq \delta_Y$ = 2·68), —CO—$CH_2$—$CH_2$—Aryl (AA′BB′ centered at 3·02), 4 OMe (3·62, 3·68, 3·77, 3·77), 3 arom. H (6·50–6·75), 1 arom. H (s, 6·94).

*Hexahydroflemingin C tetramethylether enolacetate.* 100 mg of the preceding compound in 5 ml $Ac_2O$ and 5 ml toluene were refluxed 12 hr with 100 mg *p*-toluenesulfonic acid. Addition of water and extn. with ether gave the product, IR: 5·70 (neat); NMR ($CCl_4$): $Me_2CH$— (0·89, d of 6 Hz), 1Me—C(—)—O— (1·26), 1MeCO— (2·17), 4 OMe (3·64, 3·70, 3·73, 3·77), Aryl—$CH_2$—CH═ (2 H, d, 3·34; 1 H, t, 5·82; $J$ = 7·5), 4 arom. H (6·5–7·0).

*Quinone* XII. 20 mg of hexahydroflemingin C in 1·5 ml AcOH were treated slowly with 13 mg $K_2Cr_2O_7$ in 1 ml of water. A red ppt was sepd. and crystd. from $CHCl_3$: m.p. 215–216° (Kofler); UV ($CHCl_3$): 288, 352 (ε 15,600, 9050); IR: 3·0 (OH), 6·05 (CO), 6·15. NMR ($CDCl_3$/DMSO-$d_6$): $Me_2CH$— (0·93, d of 6 Hz), 1Me—C(—)—O— (1·38), Aryl—$CH_2$—$CH_2$—CO— (4H, center at 3·0), —$CH_2CH_2$— (chromane, two approx. triplets of ca. 7 Hz, 1·9 and 2·7), 4 arom. H (6·5–7·2), 1 chel. OH (12·60).

*Homoflemingin* (IV) has m.p. 160–162° benzene–hexane. (Found: C, 70·80; H, 6·84. $C_{26}H_{30}O_6$ requires: C, 71·21; H, 6·90%); UV: 272, 317, 404 (ε 11,800, 17,400, 20,800).

*Homoflemingin triacetate* (XV). This was prepared as described for XIII, and purified by chromatography with hexane–AcOEt (4:1), m.p. 139–140°. (Found: C, 67·91; H, 6·17. $C_{32}H_{36}O_9$ requires: C, 68·07; H, 6·43%); UV: 312, 395 (ε 29,800, 8900); NMR ($CDCl_3$): Me—C═CH— (d, 1·58, 1·65, 1·77, $J_{all} \sim 1$ Hz), ═C—$CH_2$—$CH_2$—C═ (4H, center at 2·0), 3 MeCO— (2·33, 2·33, 2·37), 1 OMe (s, 3·82), Aryl—$CH_2$—CH═ (d, 3·34, $J = 7$), 2 CH═ (m, 4·9–5·3), 3 arom. H (decept. simple ABX, $\delta_A = \delta_B = 7{\cdot}15$; $\delta_X = 7{\cdot}44$), 1 arom. H (s, 7·16), CH═CH (7·43, 7·84, $J = 15{\cdot}5$), 1 chel. OH (s, 12·82).

*Ozonolysis of* XV was achieved on 100 mg as described before. The crude product contained 2,5-diacetoxybenzaldehyde, identified by TLC comparison with an authentic sample.

*Barium hydroxide degradation of homoflemingin.* 200 mg of IV and 0·5 g $Ba(OH)_2$ in 50 ml water were refluxed 3 hr under $H_2$ atmosphere; $CO_2$ was then bubbled through the reaction mixture, which was extd. with ether. The residue after evapn. of the ether was chromatographed with hexane, to give XVII, m.p. 107° (hexane). MS: *m/e* 318 ($M^+$, $C_{19}H_{26}O_4$), 303 ($M^+$—Me), 300 ($M^+$—$H_2O$), 275 ($M^+$—$C_3H_7$, $M^+$—MeCO), 249 ($M^+$—$C_5H_9$), 195 ($M^+$—$C_9H_{15}$), 123, 69, 43 (base peak, $MeCO^+$); UV: 237sh, 289, 346 (ε 11,400, 12,550, 7050); UV (NaOH 0·5N): ~252, 350 (ε ≃6750, 16,000); NMR ($CCl_4$): 3 MeC═CH (d, 1·57, 1·62, 1·77, $J_{all} \pm 1$ Hz), 1MeCO— (2·51), 1 OMe (3·87), Aryl—$CH_2$═CH (d of 7 Hz, 3·32), 2 CH═ (m, 4·9–5·3), 1 arom. H (s, 6·83), 2OH (6·13), 1 chel. OH (12·73). Subsequent elution with hexane–AcOEt gave *6-hydroxycoumarin*, m.p. 251°, blue fluorescence in UV; UV: 226, 280, 350 (ε 21,200, 10,600, 3950).[16] Methylation of XVII with $CH_2N_2$ afforded XIX, oil, UV: 278, 350 (ε 7650, 3050), NMR ($CCl_4$): 3Me—C═CH (1·57, 1·63; 1·71), 1Me—CO— (2·51), ⩥—$CH_2CH_2$—⩥ (center at 1·98), 2OMe (3·78, 3·85), Ar$\underline{CH}_2$═CH— (d, 3·30, $J = 7{\cdot}5$), 2 CH═ (m, 4·8–5·4), 1 arom. H (6·93), 1 chel. OH (12·40).

*3-Geranyl-2,4,5-trihydroxyacetophenone* (XVIII). 360 mg of 2,4,5-trihydroxyacetophenone and 450 mg of geraniol were refluxed 48 hr in 10 ml decalin. Evapn. and chromatography with hexane–AcOEt (9:1) gave a fraction, which, crystd. from hexane, afforded XVIII, m.p. 127–130°. (Found: C, 71·39; H, 7·69. $C_{18}H_{24}O_4$ requires: C, 71·02; H, 7·95); UV: 238sh, 289, 353 (ε 7350, 8870, 5050). Methylation with $CH_2N_2$ gave XIX, identical in UV, IR and NMR spectra with the same compound obtained from the degradation product of homoflemingin.

*3-Geranyl-2,4-dihydroxyacetophenone* (XX). To 5 g 2,4-dihydroxyacetophenone in 100 ml dry benzene were added with stirring 2 moles/mole of BuLi. The mixture was stirred 1·5 hr, then treated with 6 g geranyl bromide and refluxed overnight. A few drops EtOH were added to destroy excess BuLi, then the mixture was acidified and extd. with ether. The residue after evapn. was extd. with warm hexane. The residue, crystd. from hexane, gave 1·5 g XX, m.p. 120–121°. (Found: C, 74·00; H, 8·31. $C_{18}H_{24}O_3$ requires: C, 74·97; H, 8·39%); UV: 218, 233sh, 288, 320sh (ε 19,900, 10,500, 14,700, 5800); UV (NaOH 0·5 N): 251, 335 (ε 10,200, 25,000); NMR ($CCl_4$): 3 Me—C═CH (1·60, 1·67, 1·82), ⩥—$CH_2CH_2$—⩥ (center at 2·09), 1 MeCO— (2·54), Aryl—$C\underline{H}_2CH$═ (d, 3·43, $J = 7{\cdot}5$), 2 CH═ (m, 4·8–5·4), 1 OH (6·05), 2 arom. H (2d, 6·31, 7·50, $J_{ortho} = 8{\cdot}8$), 1 chel. OH (13·10).

The fraction soluble in hexane was chromatographed with hexane–AcOEt (4:1) to give 0·7 g of XX and 0·15 g *5-geranyl-2,4-dihydroxyacetophenone* (XXI), first eluted in the chromatography, m.p. 88° from hexane. (Found: C, 74·15; H, 8·31. $C_{18}H_{24}O_3$ requires: C, 74·97; H, 8·39%); UV: 236, 281, 329 (ε 12,400, 12,800, 6900); UV (NaOH 0·5 N): 252sh, 331 (ε 10,350, 14,400); NMR ($CCl_4$): 3MeC═CH (1·58, 1·65, 1·69), ⩥—$CH_2CH_2$—⩥ (center at 2·04), 1MeCO— (2·48), Aryl—$CH_2$—CH═ (d, 3·20, $J = 7{\cdot}5$), 2 CH═ (m, 4·8–5·4), 2 *para* arom. H (6·25, 2·30), 1 OH (7·52), 1 chel. OH (12·41).

*2-Hydroxy-3-geranyl-4-methoxyacetophenone* (XXII). 0·7 g of XX in 20 ml acetone were treated with 0·4 ml $Me_2SO_4$ (1 mole/mole) and 2 g $K_2CO_3$ and refluxed overnight. After filtration, evapn., and addition of water, acidification and extn. with ether, XXII was obtained. UV: 220, 237 sh, 285, 320 sh (ε 12,300, 9650, 14,400, 6750). NMR ($CCl_4$): 3Me—C═CH (1·55, 1·61, 1·75), ⩥—$CH_2CH_2$—⩥ (center at 1·9), 1MeCO— (2·43), Aryl—$C\underline{H}_2$—CH═ (d, 3·26, $J = 7{\cdot}5$), 2 CH═ (m, 4·8–5·3), 2 arom. H (2d, 6·28, 7·42, $J_{ortho} = 8{\cdot}8$), 1 chel. OH (12·55).

*2,5-Dihydroxy-3-geranyl-4-methoxyacetophenone* (XVI). To 1·15 g XXII dissolved in 25 ml pyridine and 20 ml 6% NaOH were added dropwise in 3 hr 2 g $K_2S_2O_8$ in 50 ml water. 24 hr later, the soln was extd. with ether, added with 20 ml conc. HCl and 150 ml $CHCl_3$, boiled 20 min, cooled, and extd. with $CHCl_3$.

Evapn. and crystn. from hexane gave 120 mg XVI, m.p. 69–70°, yellow fluorescent in UV light. (Found: C, 71·61; H, 8·52. $C_{19}H_{26}O_4$ requires: C, 71·67; H, 8·23%); UV: 235sh, 276, 362 (ε 10,300, 8650, 5200); UV (pH 10): 287, 415 (ε 9300, 4400); NMR ($CCl_4$): 3Me—C=CH (1·55, 1·60, 1·75), $\diagdown\!\!\diagup$—$CH_2CH_2$—$\diagdown\!\!\diagup$ (center at 2·13), 1Me—CO— (2·50), 1 OMe (3·80), Aryl-$CH_2$CH= (d, 3·30, $J$ = 7), 2 CH= (m, 4·8–5·3), 1 arom. H (s, 7·03), 1OH (5·27), 1 chel. OH (12·22).

2,4-*Dihydroxy-3-geranyl-5-methoxyacetophenone* (XVII). 2·65 g of XXIII were treated with geranyl bromide as described under XX. Working up and chromatography with hexane–AcOEt (4:1) gave 0·32 g XVII, m.p. 105–108° hexane. (Found: C, 71·66; H, 7·69. $C_{19}H_{26}O_4$ requires: C, 71·67; H, 8·23%); UV: 237sh, 290, 349 (ε 10,100, 11,400, 6600); UV (NaOH 0·5N): 250sh, 350 (ε 9350, 19,600).

*Acknowledgements*—We are indebted to Prof. E. Francini Corti, University of Florence, for botanical identification of Wars, to Dr. U. Ruggini, holder of a Donegani fellowship, for helpful work on extraction and isolation of the pigments, to Dr. A. L. Segre, Istituto di Chimica Industriale del Politecnico, Milano, for the 100 MHz NMR spectrum, and to Dr. A. Selva for mass spectra.

## REFERENCES

[1] T. Pavolini and F. Gambarin, *Agrochimica* **2**, 88 (1957) and Refs quoted.
[2] A. G. Perkin, *J. Chem. Soc.* **73**, 660 (1898).
[3] G. B. Marini-Bettòlo and A. Ballio, *Gazz. Chim. Ital.* **76**, 410 (1946).
[4] F. M. Dean, *Naturally Occurring Oxygen Ring Compounds* p. 288. Butterworths, London (1963).
[5] L. Jurd, in *The Chemistry of Flavonoid Compounds* (Edited by T. A. Geissman) p. 144. Pergamon Press, Oxford (1962).
[6] R. J. Abraham and H. J. Bernstein, *Canad. J. Chem.* **39**, 216 (1961).
[7] L. M. Jackman, in *Progress in the Chemistry of Organic Natural Products* (Edited by L. Zechmeister) Vol. 23; p. 354. Springer-Verlag
[8] C. F. Garbers, H. Schmid and P. Karrer, *Helv. Chim. Acta* **37**, 1336 (1954).
[9] H. Mayer, P. Schudel, R. Rüegg and O. Isler, *Ibid.* **46**, 963 (1963).
[10] D. J. Ringshaw and H. J. Smith, *Chem. & Ind.* 1383 (1965).
[11] G. Cardillo, L. Merlini and R. Mondelli, *Tetrahedron Letters* in press.
[12] [a] Y. Gaoni and R. Mechoulam, *Chem. Comm.* 20 (1966); [b] U. Claussen, F. v. Spulak, and F. Korte. *Tetrahedron* **22**, 1477 (1966).
[13] P. Yates, P. J. Kamarkar, D. Rosenthal, G. H. Stout, V. F. Stout, *Tetrahedron Letters* 1623 (1963); W. D. Ollis, M. V. J. Ramsay, I. O. Sutherland and S. Mongkolsuk, *Tetrahedron* **21**, 1453 (1965).
[14] R. Gompper, *Angew. Chem.* **76**, 410 (1964).
[15] Y. Ishii, *J. Agr. Chem. Soc. Japan* **26**, 510 (1952); *Chem. Abstr*, **49**, 5463 (1955).
[16] A. Mangini and R. Passerini, *Gazz. Chim. Ital.* **87**, 243 (1957).