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CHEMICAL STUDIES ON THE ORIENTAL PLANT DRUGS—XXIII[1]

PAEONIFLORIN, A GLUCOSIDE OF CHINESE PAEONY ROOT[2]

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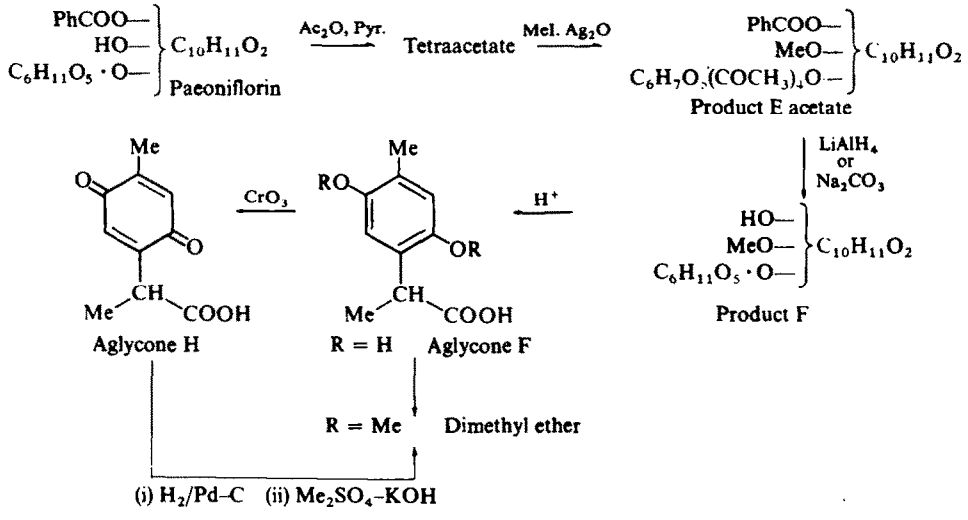
Abstract—Paeoniflorin a main principle of *Paeonia albiflora* Pallas was isolated, and identified as I, a novel glucoside of a monoterpene of the pinane series.

IN PREVIOUS reports,[4,5] a partial structure for paeoniflorin, a glucoside from *Paeonia albiflora* Pallas was proposed, and a series of reactions were described which led to an aglycone (aglycone F) whose methyl ether was proved synthetically to be DL-2(2.5-dimethoxy-4-methyl)phenylpropionic acid.

CHART 1



Since direct acid or enzymatic hydrolysis of paeoniflorin failed to yield the genuine aglycone, the aglycone F, which retains all the ten C atoms corresponding to the aglycone moiety of paeoniflorin was regarded to be a key compound for the elucidation of paeoniflorin structure. However, as the acid hydrolysis of product F to yield aglycone F involves a complex rearrangement as revealed by the aromatic nucleus

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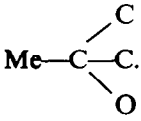
and a carboxyl group in aglycone F, which do not exist in the structure of the original glucoside, it can not provide any conclusive evidence for the structure of paeoniflorin.

Recent investigations which lead to the structure of paeoniflorin (I) are described in the present paper.

A comparison of the NMR spectra of paeoniflorin tetra- and penta-acetate has revealed that paeoniflorin contains eleven O atoms in the molecule: six in the glucose moiety, two in the benzoyloxy grouping, one as an alcoholic OH and two as ether linkages in the aglycone moiety. A singlet Me signal at τ 8·67 in the NMR spectrum of paeoniflorin tetraacetate suggests that this Me is situated in the system of



Me—C—C. The oxygen is ethereal and not a free OH, because further acetylation of paeoniflorin tetraacetate does not shift the Me signal.

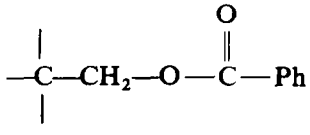
In the NMR spectrum of paeoniflorin tetraacetate, the signals (12H) in the region of τ 8·03–7·95 are assigned to four acetyl groups τ 6·34 to C_5–H,* τ 5·87 (2H) to C_6–H, τ 5·3–4·8 (4H) to C_1–C_4–H and the signals τ 2·65–1·95 (5H) to the aromatic protons in the benzoyl grouping. A singlet at τ 5·31 is assigned to an OH as it disappears on acetylation.

As acetylation of paeoniflorin tetraacetate gives no remarkable shift of the proton signal adjacent to the oxygen function, the above OH must be tertiary. This OH can be methylated to product E acetate. $C_{32}H_{38}O_{15}$, m.p. 123–125°, with diazomethane in ether–ethanol or with *p*-toluenesulphonic acid in methanol (*vide infra*), suggesting that this OH has an hemiketal or an *ortho*-ester nature.

The location of the benzoyl group in the paeoniflorin molecule has been proved by the following NMR spectral analysis.

The NMR signals at τ 5·52 (2H, s) in paeoniflorin tetraacetate; τ 5·50 (2H, AB Type) in paeoniflorin pentaacetate; τ 5·45 in product E acetate; $\delta_{aq.}$ +0·75 ppm (2H, s) in product A_1; and $\delta_{aq.}$ +0·68 (2H, s) in product A_2 can be assigned to the methyleneoxy grouping. A typical AB type doublet ($\tau_A - \tau_B = 0{\cdot}19$ ppm, $J_{AB} = 12$ c/s) of methylene is observed in XV, which is described later. Product F acetate shows an upfield shift of the methylene signal (τ 5·72) in contrast to that in product E acetate (τ 5·45). This is caused by replacement of the benzoyl by acetyl, suggesting that the benzoyl is at the position neighbouring the methyleneoxy grouping.

The NMR signal of the methyleneoxy group always appears as an AB type or as a singlet pattern, which suggests that this group is attached to a tertiary C atom. Thus the benzoyl group in paeoinflorin exists in the following system:



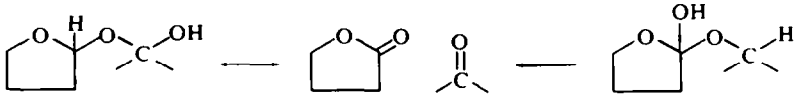
The benzoyl grouping cannot be present in the sugar moiety of paeoniflorin, since 2,3,4,6-tetra-O-methyl-D-glucose is formed on hydrolysis of paeoniflorin permethyl ether prepared by the methylation of paeoniflorin.[6] A characteristic singlet signal (1H) at τ 4·60 is observed in the NMR spectrum of paeoniflorin tetra-

* C_1–C_6 refers the carbons in the glucose moiety of the glucosides.

acetate, and the corresponding signal also appears in paeoniflorin pentaacetate, product E acetate, and product F acetate. This signal disappears when paeoniflorin tetraacetate is oxidized with chromium trioxide in acetic acid, the product being a keto-lactone, (XI), $C_{31}H_{34}O_{15}$, m.p. 148°, $[\alpha]_D$ −53·9°. The presence of a keto group was proved by formation of a 2,4-dinitrophenylhydrazone, $C_{37}H_{38}N_4O_{18}$, m.p. 206° (dec), and ethyleneketal, $C_{33}H_{38}O_{16}$, m.p. 170°, while the γ-lactone system is revealed by the IR absorption at around 1780 cm^{-1} (in tetrahydrofuran) in XI and its derivatives. The NMR signals of the keto-lactone (XI) are assigned as follows: τ 8·44 (3H, s) $\diagup\!\!\diagdown$C—C$\underline{H}_3$; τ 8·14, 8·07, 8·02, 8·00 (each 3H, s) 4 —OCOC$\underline{H}_3$; τ 7·85–6·60 (5H) methylene and methine protons in the aglycone part; τ 6·45 (1H, m) C_5–H; 5·91 (2H, br. d) C_6–H; τ 5·42 (2H, s) —C$\underline{H}_2$O; τ 5·3–4·9 (4H) C_1–C_4–H; τ 2·9–2·0 (5H) aromatic protons in benzoyl group.

The chromic acid oxidation of paeoniflorin tetraacetate involves the formation of two carbonyls with a loss of two hydrogens without uptake of oxygen. This fact suggests the presence of a masked ketone (ketal) or a masked lactone (*ortho*-ester) system in the starting compound, and the possible reactions can be envisaged as follows:

CHART 2

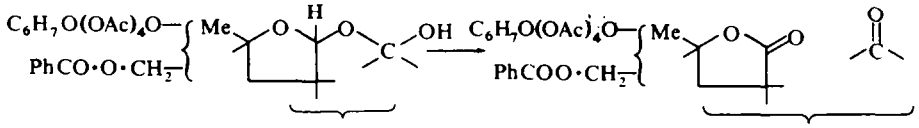


Hemiketal-acetal System | Ketolactone System | *Ortho*-ester System

The disappearance of the OH signal at τ 5·30 and a characteristic singlet (1H) at τ 4·60 in the NMR spectrum of paeoniflorin tetraacetate on chromic acid oxidation favours the hemiketal-acetal system, as the signal at τ 4·60 can reasonably be assigned to an acetal proton.

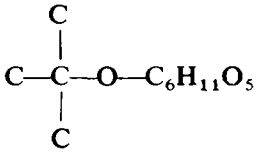
The Me signal of the ketonic lactone shows a down field shift (τ 8·44) in comparison with that of paeoniflorin tetraacetate (τ 8·64). This may be due to the carbonyl of the newly formed lactone. In which case the above partial structure may be extended to include all the oxygen functions in the aglycone part of paeoniflorin.

CHART 3



II XI

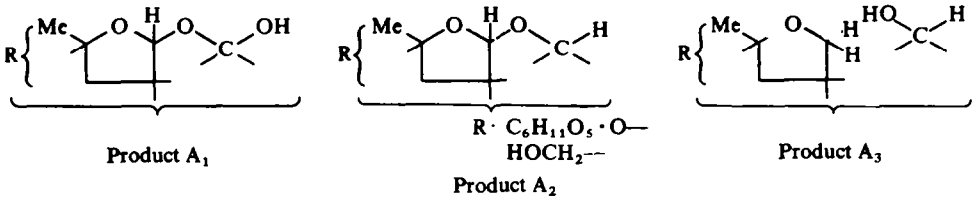
Since all the protons attached to the C atoms bearing O atoms have been assigned, the glucosyl moiety of paeoniflorin must be linked to a tertiary OH of the aglycone:



Reduction of the ketone group of the ketone-lactone (XI) with sodium borohydride in ethanol is accompanied by debenzoylation and deacetylation and affords an alcoholic compound, $C_{16}H_{24}O_{10}$ (XII), m.p. 236–238°, but if ethanol and dichloromethane (2:1) are used as solvent, the product is a compound, $C_{31}H_{36}O_{15}$ (XIII), m.p. 203°, which retains acetyl and benzoyl groups. The γ-lactone system is retained in these reduction products and shows IR absorption at 1750 cm^{-1} (KBr) and 1780 cm^{-1} ($CHCl_3$), respectively. The treatment of an aqueous solution of paeoniflorin with Amberlite IRA 400 causes debenzoylation to product A_1^*, $C_{16}H_{24}O_{10}$, m.p. 193–195°,* which may also be obtained from paeoniflorin tetra- and penta-acetates by the action of LAH. In addition to product A_1 this reduction of paeoniflorin acetates produces product A_2† and product A_3. The product A_2 (VIII), $C_{16}H_{24}O_9$, m.p. 163°, which is also obtained by the reduction of A_1 with sodium borohydride or LAH reduction of paeoniflorin tetraacetate tosylate $C_{38}H_{42}O_{17}S$, m.p. 140° (dec), is formed by replacing the hemiketal OH of product A_1 with hydrogen, since the signal of a proton attached to the C atom bearing the ether linkage appears at $\delta_{aq.}$ +1·85 ppm (1H, t) and the acetal proton at $\delta_{aq.}$ −0·81 (1H, s) ($\delta_{aq.}$ −0·76 (1H, s) in A_1) is retained.

The product A_2 yields a pentaacetate, $C_{26}H_{34}O_{14}$, m.p. 203°, whose IR spectrum shows the absence of a free OH group. The NMR spectra of product A_3 (X), $C_{16}H_{26}O_9$, m.p. 267°, and its aglycone, $C_{10}H_{16}O_4$, m.p. 214–219°, reveal the presence of one C–Me and one primary alcoholic group and the absence of an acetal proton. The partial structures of products A_1, A_2 and A_3 are formulated as follows:

CHART 4



The previously described keto-lactone (XI), when heated under reflux with aqueous Na_2CO_3, yields benzoic acid and an α,β-unsaturated keto-carboxylic acid (XIV; IR (KBr): 1730 (COOH), 1680 cm^{-1} ($\diagdown\!\!\diagup$C=O); UV λ_{max}^{EtOH}: 249 mμ); sodium salt: IR absorptions (KBr) at 1655 (α,β-unsaturated 6-membered ring C=O), 1610 (C=C), 1590 cm^{-1} (COO—); UV λ_{max}^{EtOH}: 252 mμ.

The pentaacetate (XV) of XIV, $C_{26}H_{32}O_{15}$, m.p. 182°, gives IR absorptions at 3200–2800 (COOH), 1760 ($OCOCH_3$), 1730 (COOH) and 1695 cm^{-1} (conj. C=O), and UV absorption max at 242 mμ (log ε 3·86).

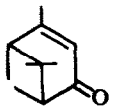
In α,β-unsaturated 6-membered monocyclic ketones, the UV max generally appears at 235 ± 5 mμ, but in bicyclic systems the UV max is in the region of 250–265 mμ [Verbenone λ_{max} 253 mμ (log ε 3·84); umbellulone λ_{max} 265 mμ (log ε 3·52)].[7] In the NMR spectrum of the acetate (XV) a cyclopropane ring system is absent while the UV max of XIV resembles that of verbenone.

On the other hand the NMR spectrum of verbenone shows a long range coupling

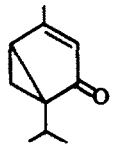
* It was previously reported as product A. m.p. 186°.

† It was described as product A′ in the previous report.[1]

CHART 5



Verbenone



Umbellulone

between the Me (τ 8·00, d, J = 1·5 c/s) and the olefinic proton (τ 4·35, q, J = 1·5 c/s). A similar coupling is present between the Me and the corresponding proton in the α,β-unsaturated pentaacetate (XV), and the chemical shift of the latter proton (τ 4·40, q, J = 1 c/s) reveals its location in the α-position of the α,β-unsaturated ketonic system.[8] Therefore the carbon skeleton of the aglycone part of paeoniflorin is most likely to be a pinane.

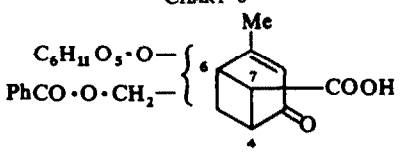
Compound XIV. on heating with sulphuric acid followed by methylation with dimethylsulphate, affords α(2,5-dimethoxy-4-methyl)phenylacrylic acid (XXI), m.p. 167°, (IR ν_{max}^{KBr} (cm^{-1}): 1700 (conjugated COOH), 1628 (double bond), identical with a synthetic sample.[5]

In contrast to the formation of aglycone F from products F and A_1, the carboxyl and the carbinol groups in the glucoside (XIV) are retained in XXI, the former as an intact and the latter as a dehydrated form.

The keto lactone (XI) on chromatography over silica gel yields a product, $C_{31}H_{34}O_{15}$, (XVII), m.p. 158–160°, retaining acetyl and benzoyl groups and having an α,β-unsaturated ketonic system.

The above experimental results supported the following partial structure of the α,β-unsaturated ketonic acid.

CHART 6



α,β-Unsaturated ketonic acid (XIV)

The tertiary OH group to which the glucosyl group is attached must be located at the 4, 6 or 7 position, but in accordance with the disposition of the hydroxyls in the aromatic aglycones (VII, XXI), the 6-position is most likely.

In the NMR spectra of paeoniflorin and its derivatives, the methyleneoxy group linked to the benzoyl group reveals a singlet or AB type doublet, so the neighbouring C_7 or C_4 atom should have no proton.

On the other hand, the acetal proton appears in the NMR spectra of these compounds as a singlet proving that the C_7 is quaternary, which is linked to the methylene group. Therefore, the α,β-unsaturated keto acid can be represented by the structure XIV.

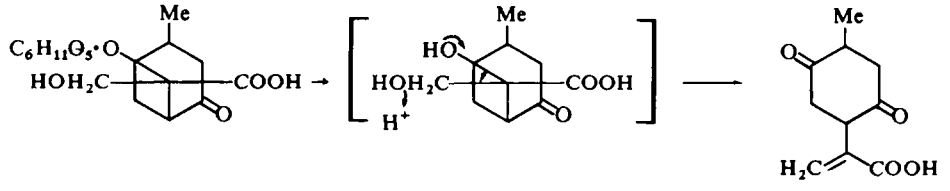
The acetate (XV) on catalytic hydrogenation with Adams' catalyst yields a dihydro derivative (XVIII), $C_{26}H_{34}O_{15}\cdot H_2O$, m.p. 219–225°, whose methyl ester, $C_{27}H_{36}O_{16}$, m.p. 169–170·5°, shows in the NMR spectrum the presence of $COOCH_3$ (τ 6·30, 3H, s), and —C(H)—CH_3 groups (τ 8·99, d, J = 6·5 c/s).

The IR spectrum of the deacetylated dihydro compound (XIX), $C_{16}H_{24}O_{10}$, m.p. 180–182°, shows the presence of a carboxyl (1725 cm^{-1}) and a 6-membered ring ketone (1710 cm^{-1}, sh). On heating XIX with dilute sulphuric acid, an optically inactive acidic compound, $C_{10}H_{12}O_4$ (XX), m.p. 134°, is produced. This is not aromatic and shows strong end absorption in the UV spectrum and IR absorptions at 1710 cm^{-1} (6-membered ring ketone), 1688 cm^{-1} (α,β-unsaturated carboxyl) and 1625 cm^{-1} (double bond).

The NMR spectrum of this compound shows a doublet signal of a C—Me at τ 8·85 (J = 5 c/s), an ABX type quartet at τ 6·20 (J_{AX} = 12·5 c/s; J_{BX} = 6·2 c/s) and singlets at τ 4·25 and τ 3·50, which correspond to a vinyl group conjugated with a carboxyl, the signal of which appears at τ 1·3. These data show a reasonable agreement with the structure XX. The weak signals accompanying the signals of methyl and vinyl protons in the NMR spectrum of the above compound suggest a contamination with the lactol form (XX'), since the IR spectrum measured in $CHCl_3$ solution shows a weak lactonic carbonyl absorption at 1787 cm^{-1}.

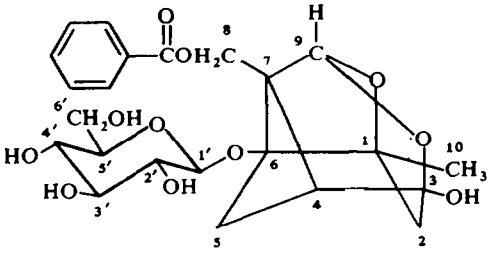
The formation of the cyclohexadione type compound has been explained by the following scheme of reactions in giving formula (XIX) for the hydrogenated keto-carboxylic acid.

CHART 7



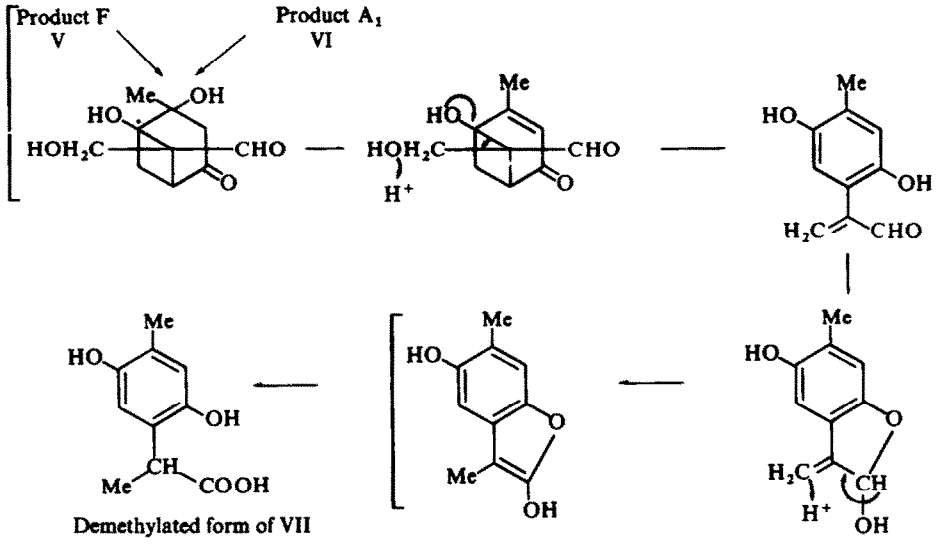
Ozonolysis of XV gives a product with UV absorption max at 290 mμ (ε = 66) and a positive iodoform reaction indicating the presence of methylketone group. On methylation with diazomethane, this product (XXII) yields a methyl ester (XXIII). $C_{27}H_{36}O_7$, whose NMR spectrum reveals two carbomethoxyls, five O-acetyls, one C-acetyl, and AB-type doublets of a methylene group. The Baeyer–Villiger oxidation of the dimethyl ester (XXIII) yields an oily substance whose IR absorption appears at 1800 cm^{-1} indicating a 4-membered ring ketone (XXIV), but the compound was not obtained in a pure state due to shortage of material. On the basis of all the evidence, paeoniflorin has the structure I, with the stereochemical features as shown below (or its antipode concerning the aglycone part). The β-glucosidic linkage in paeoniflorin is proved by the NMR spectra of paeoniflorin derivatives which give the signal of $C_{1'}$—H as doublet with J = 6–8 c/s.

CHART 8



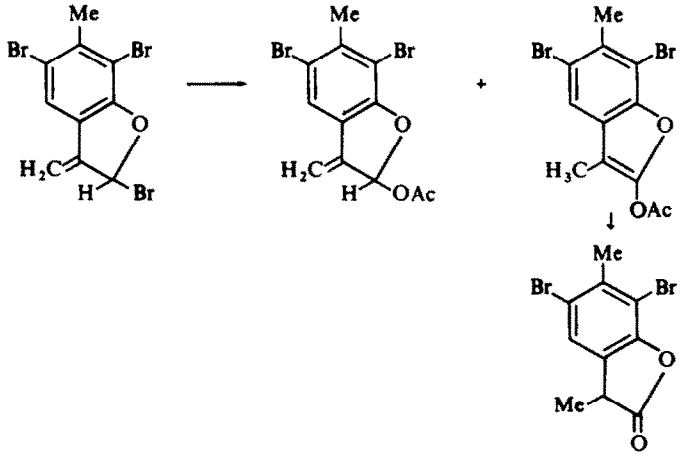
The mechanism of the respective formation of aglycone F from products F and A_1 can be elucidated as follows:

CHART 9



An analogous example is found in literature,[9] which is explained by the following reactions:

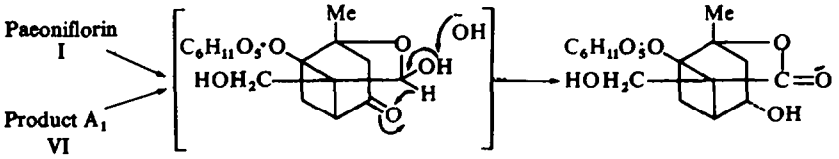
CHART 10



The benzoyl group of paeoniflorin is removed by heating in 2N Na_2CO_3 leaving a compound. $C_{16}H_{24}O_{10}$. m.p. 217°. which is also obtained from product A_1 by the same procedure. The IR spectrum of this compound shows a γ-lactone at 1750 cm^{-1}. The presence of an aldehyde or a carboxyl group is excluded by the NMR spectrum. while a ketone is rejected by the absence of UV absorption at 270–300 mμ. On acetylation a hexaacetate. $C_{28}H_{36}O_{16}$. m.p. 134°. is formed. whose NMR spectrum indicates

the absence of the characteristic acetal proton. Consequently, the product of the alkaline treatment has been formulated as XXV, and the mechanism of its formation is explained as follows:

CHART 11

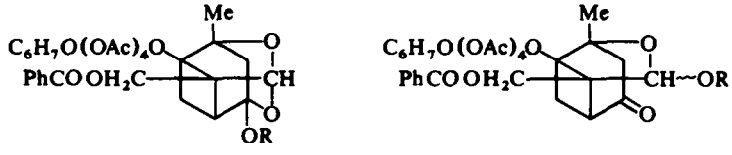


Analogous intramolecular rearrangements of this type have been reported.[8]

The alcoholic compound (XII) obtained by the reduction of the ketonic lactone (XI) is not identical with XXV, but is an epimer at the alcoholic hydroxyl.

Treatment of paeoniflorin with *p*-toluenesulphonic acid in methanol, affords two methyl ethers, product M_1 and product M_2. The respective acetates obtained in crystalline form show an alcoholic O—Me signal in the NMR spectra, and M_1-acetate, m.p. 123–125°, is identical with product E acetate. The M_2-acetate, $C_{32}H_{38}O_{15}$, m.p. 174·5°, gives IR absorption at 1715 cm^{-1} showing the presence of a 6-membered ring ketone, which yields a 2,4-dinitrophenylhydrazone, m.p. 262–264°, $C_{38}H_{42}N_4O_{18}$. The structure of M_2 has been represented by XXVI. Using ethanol as the solvent the corresponding ethyl ethers, products K_1 and K_2 are obtained. These give crystalline acetates: product K_1-acetate, colourless needles, m.p. 165·5°, $C_{33}H_{40}O_{15}$ and product K_2-acetate, colourless prisms, m.p. 153·5°, $C_{33}H_{40}O_{15}$.

CHART 12



R = Me M_1-acetate
R = Et K_1-acetate

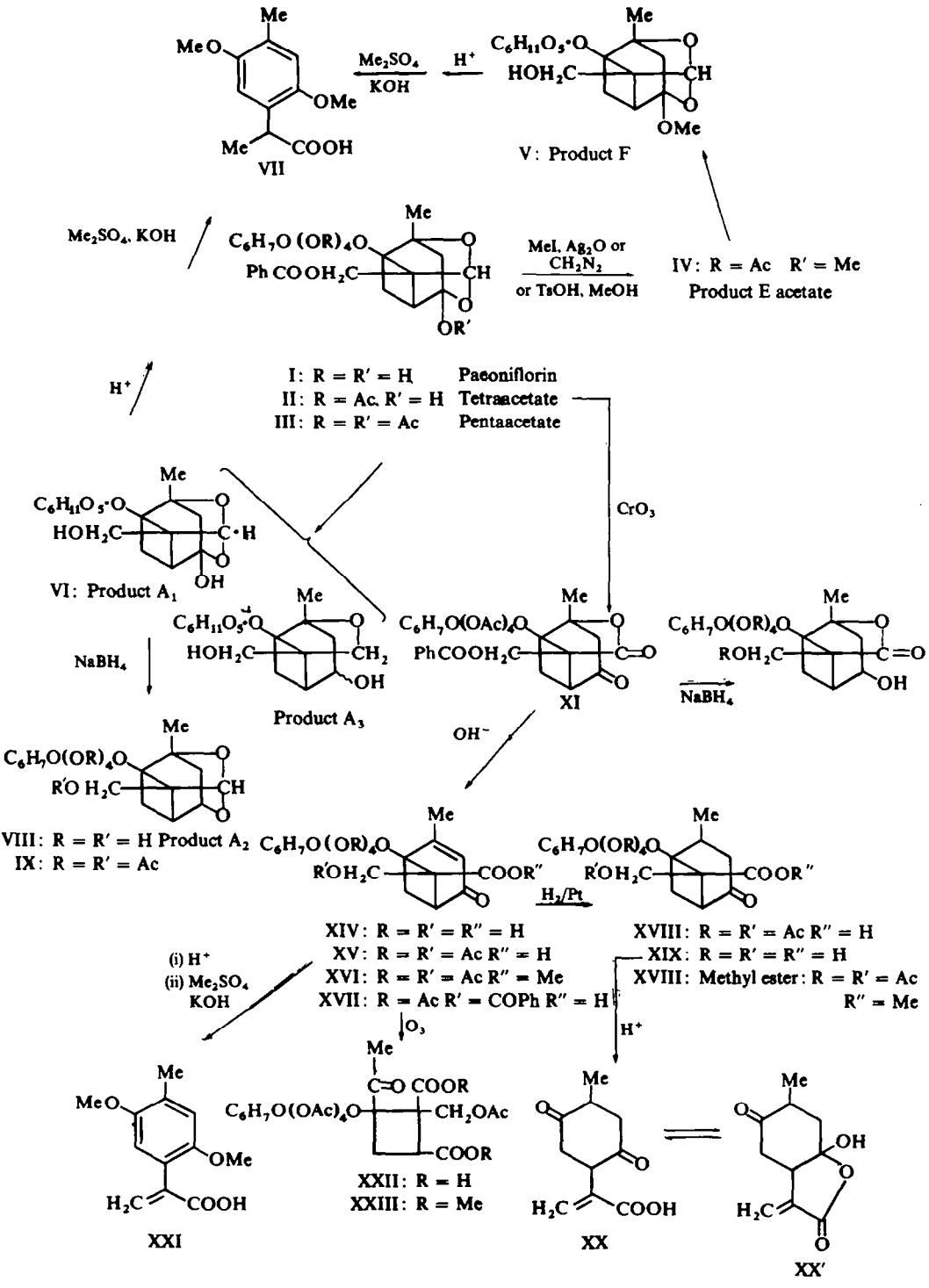
R = Me M_2-acetate
R = Et K_2-acetate

EXPERIMENTAL

M.ps were determined in a Yanagimoto m.p. apparatus and are uncorrected. Unless otherwise stated, the NMR spectra were measured in $CDCl_3$ on a Japan Electron Optics Lab. JMN-3H-60 (60 Mc), a Varian A-60 (60 Mc) or a Varian Model HA-100 (100 Mc) instruments and recorded in τ-values with TMS as the internal references. Optical rotations were measured with Yanagimoto Photo-magnetic polarimeter Model OR-20. The UV absorption spectra were measured in EtOH on a Cary Spectrophotometer Model 11. IR spectra on a Japan Spectroscopic Co. Model 301 or DS-402G spectrophotometer. The p*Ka'* value for VI was measured with an Automatic Titrator TTTlc (Radiometer, Copenhagen, Denmark).

Product E acetate (IV). To a soln of II (100 mg) in MeOH, excess CH_2N_2 in Et_2O was added. The soln was kept at room temp overnight and the solvent was evaporated *in vacuo*. Recrystallization from EtOH gave colourless needles, m.p. 123–125°, identical with product E acetate[4] (IV) by TLC (Silicagel G, Merck Co., benzene–Et_2O (1:1)), comparison of IR spectra and by a mixed m.p. The same product was obtained when EtOH was used instead of MeOH as solvent.

CHART 13


Me
MeO
OMe
Me
COOH
VII
Me2SO4
KOH
H+
C6H11O5·O
HOH2C
V: Product F
Me2SO4, KOH
C6H7O(OR)4O
Ph COOH2C
OR'
MeI, Ag2O or
CH2N2
or TsOH, MeOH
IV: R = Ac R' = Me
Product E acetate
I: R = R' = H Paeoniflorin
II: R = Ac, R' = H Tetraacetate
III: R = R' = Ac Pentaacetate
CrO3
VI: Product A1
OH
NaBH4
Product A3
C6H7O(OAc)4O
PhCOOH2C
XI
C6H7O(OR)4O
ROH2C
OH-
VIII: R = R' = H Product A2
IX: R = R' = Ac
R'OH2C
COOR"
H2/Pt
XIV: R = R' = R" = H
XV: R = R' = Ac R" = H
XVI: R = R' = Ac R" = Me
XVII: R = Ac R' = COPh R" = H
XVIII: R = R' = Ac R" = H
XIX: R = R' = R" = H
XVIII: Methyl ester: R = R' = Ac
R" = Me
(i) H+
(ii) Me2SO4
KOH
O3
C=O COOR
CH2OAc
XXII: R = H
XXIII: R = Me
XXI
XX
XX'

Hydrolysis of permethylated paeoniflorin. Paeoniflorin (I) (2 g) was dissolved in a mixture of dimethylformamide (25 ml) and MeI (9 ml), and 8·7 g of Ag_2O was added to the soln under ice cooling. The mixture was stirred in a refrigerator overnight and then filtered; $CHCl_3$ was added with the filtrate, and the crystals which separated out were filtered off. The filtrate together with H_2O (100 ml) containing 1 g of KCN was shaken with $CHCl_3$. After the usual treatment, the chloroform soln yielded a dark brown syrup which was chromatographed over silica gel (20 g). From a benzene–Et_2O (7:3) fraction, 1·2 g of faint yellow syrup was obtained which showed a single spot on TLC (Silica gel G, benzene–Et_2O (1:1); IR $\nu_{max}^{liq.}$: no OH, 1730, 1275 cm^{-1} (OBz).

The above methyl ether (40 mg) was heated in 7 ml MeOH–conc HCl (6:1) for 5 hr. The soln was diluted with H_2O and the most of MeOH was removed *in vacuo*. The soln was refluxed for 1 hr, and then identified on TLC. Using silica gel G, and solvent systems: (a) benzene–Et_2O (1:1), (b) benzene-Me_2CO–H_2O (10:10:1, upper layer, detection: Aniline hydrogen phthalate) the methylated sugar was proved to be 2.3.4.6-tetra-O-methylglucose.

Keto-lactone (XI). To a soln of II (7 g) in AcOH (75 ml), a soln of CrO_3 (1 g) in AcOH (10 ml) was added dropwise under cooling with water. After standing at room temp overnight, a small amount of MeOH was added, and the mixture poured into ice water. A ppt separated and after drying in a desiccator, it was chromatographed over silic acid (Mallinckrodt) (30 g) yielding colourless prisms from the CH_2Cl_2 fraction. Recrystallization from MeOH gave colourless prisms (XI), m.p. 148°, $[\alpha]_D^{29}$ −53·9 (c = 1·02, EtOH), yield 3·7 g; IR ν_{max}^{Nujol}: no OH, 1780–1700 (γ-lactone, OAc, OBz, ketone). IR ν_{max}^{THF}: 1780 (γ-lactone); 1760 and 1740 (sh) (OAc and OBz); 1710 (sh) (ketone); UV λ_{max}: 229, 267 (sh), 272, 280 mμ (R—O, CO—Ph). (Found: C, 57·42; H, 5·47. $C_{31}H_{34}O_{15}$ requires: C, 57·58; H, 5·30%).

From the MeOH fraction of the above chromatography, colourless crystals (XVII, 50 mg), m.p. 160°, were obtained. Its UV spectrum showing absorption maxima at 231 mμ (log ε 4·33), 257 mμ (sh) (log ε 3·26) and 281 mμ (sh) (log ε 3·08) and absorption minimum at 212 mμ (log ε 3·96) revealed the presence of a benzoyl ester and an α,β-unsat. ketonic system; NMR: 7·99 (12H, s) and 7·90 (3H, s) (4—OAc, CH_3—C=C): 5·30 (2H, unresolved AB type doublets, $C\underline{H}_2$—OBz); 4·42 (1H, unresolved q, J = ca 1 c/s H—C=C); 3·69 (1H, br. COOH) 2·70–1·95 (5H, OBz). (Found: C, 56·81; H, 5·47. $C_{31}H_{34}O_{15}\frac{1}{2}H_2O$ requires: C, 56·79; H, 5·38%).

2,4-Dinitrophenylhydrazone of keto-lactone (XI). By the usual procedure, XI gave a 2,4-dinitrophenylhydrazone, yellow needles from $CHCl_3$–MeOH (1:1), m.p. 206° (dec); IR ν_{max}^{THF} cm^{-1}: 1789 (γ-lactone); 1770–1750 (OAc); 1737 (OBz). (Found: C, 53·54; H, 4·58; N, 6·75. $C_{37}H_{38}N_4O_{18}$ requires: C, 53·75; H, 4·60; N, 6·78%).

Ethylene ketal of keto-lactone (XI). To soln of XI (300 mg) in AcOH (5 ml), ethylene glycol (0·9 ml) and BF_3-etherate (0·6 ml) were added. The mixture was stirred for 1·5 hr at room temp and poured into ice water. A white powder separated was crystallized from EtOH to give colourless needles (200 mg), m.p. 170°; IR ν_{max}^{THF} cm^{-1}: 1788 (γ-lactone); 1770–1750 (OAc); 1737 (OBz). (Found: C, 57·38; H, 5·38; $C_{33}H_{38}O_{16}$ requires: C, 57·39; H, 5·51%).

Reduction of the keto-lactone (XI) *with* $NaBH_4$ (1). The keto-lactone XI (1 g) was dissolved in 50 ml EtOH, and 500 mg of $NaBH_4$ was added with stirring. After 4 hr stirring at room temp, a few drops of AcOH were added, and the soln was diluted with water, but no ppt was formed. The soln was deionized with Amberlite IR 120 (H form) and IR 4B (OH form) resins, and the solvent was evaporated *in vacuo*. Treatment with MeOH gave crude crystals (210 mg), which were recrystallized from MeOH to give 120 mg of colourless plates (XII), m.p. 236–238°; IR ν_{max}^{KBr}: 3600–3100 (OH); 1750 (lactone). NMR (60 Mc, in D_2O, TMS as external reference): 8·46 (3H, s. >C—CH_3); 6·19 (2H, br. d, C_6—H_2); 5·96 (2H, s, —$C\underline{H}_2OH$); 5·66 (1H, m); 5·32 ($\underline{H}OD$ + C_1-H). (Found: C, 51·29; H, 6·47. $C_{16}H_{24}O_{10}$ requires: C, 51·06; H, 6·43%).

(2) To a soln of XI (500 mg) in 15 ml EtOH–CH_2Cl_2 (2:1), 100 mg $NaBH_4$ was added by portions. After stirring for 1·5 hr at room temp, a small amount of AcOH was added. The reaction mixture was diluted with CH_2Cl_2, washed with H_2O and dried over Na_2SO_4. Evaporation of the solvent followed by crystallization from EtOH gave 310 mg of XIII, as colourless needles, m.p. 203°; IR $\nu_{max}^{CHCl_3}$: 3500 (OH), 1780 (γ-lactone), 1750 (OAc), and 1725 (OBz); NMR: 8·55 (3H, s. >C—CH_3) 8·19, 8·05, 8·01, 7·96 (each 3H, s, 4 —OAc) 2·65–1·90 (5H, Ar · H). (Found: C, 57·04; H, 5·38. $C_{31}H_{36}O_{15}$ requires: C, 57·41; H, 5·51%).

Product A_1 (desbenzoyl paeoniflorin) (IV). (1) To a soln of I (1 g) in H_2O (10 ml). 10 ml Amberlite IRA 400 resin (OH form) was added. The mixture was stirred for 1·5 hr at room temp. filtered from the resin and the solvent was evaporated *in vacuo*. Treatment of the residue powder with EtOH gave 210 mg of crude VI which yielded colourless prisms (130 mg) after recrystallization from MeOH. m.p. 193–195°. $[\alpha]_D^{29}$ −33·2 (c = 0·98. H_2O) identical with product A_1[4] by TLC (Silica gel G. $CHCl_3$–MeOH (3:1)). a comparison of their IR spectra and a mixed m.p. it showed pKa' 11·5.

(2) Paeoniflorin I (1 g) was dissolved in abs MeOH (6 ml) containing 1 ml of 0·1N NaOMe. After heating under reflux for 30 min. the filtrate was treated with Amberlite IR 120 resin (H form). and concentrated *in vacuo*. Dilution with H_2O followed by removal of benzoic acid by extraction with Et_2O. and evaporation of the solvent gave 70 mg of VI after crystallization from MeOH.

(3) A soln of I (1 g) in H_2O (20 ml) was treated with 0·33 g of $Ba(OH)_2 \cdot H_2O$. and the mixture was allowed to stand at room temp for 2 days and after treatment as in the above experiment gave 200 mg of VI.

Product A_2 (VIII). Compound VI (1·4 g) was dissolved in H_2O (10 ml) and a soln of $NaBH_4$ (200 mg) in H_2O (5 ml) was added. After stirring the mixture at room temp for 1 hr. a few drops of AcOH were added. and the solvent was evaporated *in vacuo*. The residue redissolved in water. was treated with Amberlite IR 120 (H form) and subsequently with Amberlite IRA 400 resin (OH form). The solvent was removed *in vacuo* to give a glassy residue. from which a crude product A_2 (VIII: 850 mg) was obtained and after recrystallization from EtOH. as colourless prisms (600 mg). m.p. 163°. $[\alpha]_D^{29}$ −25·5 (c = 1·1. H_2O): IR ν_{max}^{KBr}: 3600–3200 (OH): no carbonyl. (Found: C. 53·69: H. 6·65. $C_{16}H_{24}O_9$ requires: C. 53·33: H. 6·71%).

Product A_2 acetate (IX). Acetylation of VIII by the usual method gave a pentaacetate. m.p. 203–204°. which was identical with the acetate of product A_1[4] by a mixed m.p. and comparison of their IR spectra: IR ν_{max}^{Nujol} cm^{-1}: no OH: 1750 (OAc): NMR: 8·72 (3H. s. $\diagdown$C—CH_3): 8·00–7·86 (15H. 5 × OAc): 6·62 (1H. s. acetal–H). (Found: C. 54·42: H. 5·82. $C_{26}H_{34}O_{14}$ requires: C. 54·74: H. 5·96%).

Paeoniflorin tetraacetate tosylate. Compound II (500 mg) was dissolved in pyridine (25 ml) and 1 g of tosyl chloride was added. The soln was allowed to stand at 25° for 10 days. and then poured into ice water. The gummy ppt was crystallized from EtOH to give colourless needles. m.p. 140° (dec). 190 mg: IR ν_{max}^{KBr}: no OH: 1750 (OAc): 1720 (sh) (OBz): 1600. 1370. 1180. 930. 830. (Found: C. 57·12: H. 5·34: S. 4·12. $C_{38}H_{42}O_{17}S$ requires: C. 56·85: H. 5·27: S. 3·99%).

LAH reduction of the tosylate. To a suspension of LAH (300 mg) in abs. THF (100 ml). a tetrahydrofuran soln of the above tosylate (170 mg) was added dropwise. After refluxing for 4 hr. water was added and the ppt was removed by centrifuging. Deionization with Amberlite IR 4B (OH form) and IR 120 (H form) resins followed by evaporation of the solvent gave an amorphous powder. Acetylation of this product using Ac_2O and pyridine at room temp afforded colourless needles (from EtOH). which were identical with product A_2 acetate (IX).

LAH reduction of paeoniflorin tetraacetate (II). Compound II (6·6 g) was dissolved in abs THF (100 ml). and LAH (3 g) suspended in the same solvent was added dropwise with stirring and ice cooling. The reaction mixture was stirred at room temp for 6 hr. and refluxed for 4 hr. Water was added to the soln and the ppt was removed by centrifuging. The soln was deionized by Amberlite IR 120 (H form) resin. and the solvent was evaporated *in vacuo* to afford a foamy residue (3·4 g). Crystallization from EtOH gave 1·25 g of VI. m.p. 197°. Product A_2 (VIII). m.p. 162–163°. (0·15 g) was obtained from the mother liquor of VI. A small amount of product A_3 was deposited from the mother liquor on standing. which was recrystallized from MeOH–Et_2O to afford colourless prisms. m.p. 267°. IR spectrum showed no carbonyl absorption. (Found: C. 52·99: H. 7·46. $C_{16}H_{26}O_9$ requires: C. 53·04: H. 7·23%).

Aglycone A_3. Product A_3 (100 mg) was subjected to hydrolysis in 7% HCl (10 ml) by heating for 2 hr on a boiling water bath. Upon cooling. the soln was washed with Et_2O. No aglycone was isolated from the ethereal layer. The water layer was deionized by Amberlite IR 4B (OH form). and removal of the solvent *in vacuo* gave a syrupy residue. The aglycone was separated from glucose by cellulose powder column (5 g) using BuOH saturated with water for elution. Recrystallization from MeOH–Et_2O gave colourless prisms (13 mg). m.p. 214–219°. IR spectrum showed no carbonyl or double bond absorption. It reduces neither Fehling nor Tollens reagent. Mass spectrum: m/e 182 (M—H_2O): NMR (D_2O) δ_{aq}: 3·49 (3H. s) ($\diagdown$C—CH_3). 3·28–2·03 (5H). 0·98 (1H. d) and 0·23 (1H. d. J = 9·5 c/s) (—CH_2—O—) 0·88 (2H. s) (—$C\underline{H_2}$—OH). (Found: C. 60·06: H. 7·93: $C_{10}H_{16}O_4$ requires: C. 59·98: H. 8·05%).

α,β-Unsaturated ketocarboxylic acid (XIV). Compound XI (3 g) was suspended in 2N Na_2CO_3 (60 ml). and heated on a boiling water bath. The clear soln was heated under reflux for another 2 hr. Amberlite

IR 120 (H form) resin was added after cooling. and the soln passed through a column of the same resin. and the elute concentrated *in vacuo*. After removal of benzoic acid by extraction with Et_2O. the solvent was evaporated *in vacuo*. to give a colourless glassy powder (XIV). UV λ_{max} 249 mμ; IR ν_{max}^{KBr} cm^{-1}: 1730 (COOH); 1680 (C=O). This compound was submitted to acetylation without further purification.

α,β-Unsaturated keto-carboxylic acid acetate (XV). Compound XIV was acetylated using pyridine (10 ml) and Ac_2O (10 ml). After standing 24 hr at room temp. the mixture was poured into ice water. but no ppt separated out. After washing with Et_2O. the water layer was acidified with dil HCl to afford colourless plates. On recrystallization from EtOH. XV was obtained as colourless needles (1·6 g). m.p. 182°. $[\alpha]_D^{29}$ −26·2 (*c* = 0·94. EtOH); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH; 3200–2800. 1730 (COOH). 1760 (OAc). 1695 (α,β-unsat. ketone); UV λ_{max} 242 (3·86) mμ. (Found: C. 53·24; H. 5·53. $C_{26}H_{32}O_{15}$ requires: C. 53·42; H. 5·52%).

Sodium salt of α,β-unsaturated keto-carboxylic acid. In the preceding experiment the Na salt of XIV was obtained when ion exchange resin was applied by a batch process. It was a crystalline powder which shows IR absorption (KBr) at 1665 (α,β-unsat. ketone); 1610 (C=O); 1590 (COOH) cm^{-1} and UV absorption max at 252 mμ.

α-(2.5-Dimethoxy-4-methyl)phenyl acrylic acid (XXI). Compound XV (1·05 g) was suspended in a mixture of MeOH (10 ml) and 2N Na_2CO_3 (20 ml). and was refluxed for 3 hr on a boiling water bath. Deionization with Amberlite IR 120 (H form) resin and washing with Et_2O followed by evaporation of the solvent *in vacuo* gave a syrupy residue (XIV).

This was dissolved in 2N H_2SO_4 (6 ml) and heated on a boiling water bath. The aglycone separated out and was extracted with Et_2O three times at intervals of about 30 min during the course of the reaction. The combined Et_2O soln was washed with H_2O. dried over Na_2SO_4. and the solvent was evaporated yielding a yellowish brown residue. This was dissolved in MeOH (20 ml) containing 2 ml of Me_2SO_4 and the soln heated under reflux. Finely powdered KOH (2 g) was added at once and the soln was refluxed for another 1 hr. After cooling. the soln was diluted with water. and MeOH was removed under reduced press. The aqueous soln was acidified with dil H_2SO_4. and the product was extracted with Et_2O. The acidic component was extracted with dil $NaHCO_3$ from the Et_2O soln and usual treatment gave yellowish brown syrup. which was chromatographed over silicic acid (20 g). The $CHCl_3$–acetone (95:5) fraction was subjected to chromatography using another column of the same absorbent. and from the $CHCl_3$ fraction about 20 mg of crude crystals was obtained. Recrystallization from $CHCl_3$–n-hexane afforded micro needles. m.p. 167°. which were identical with an authentic specimen of α-(2.5-dimethoxy-4-methyl)-phenyl acrylic acid[2] by a mixed fusion. and comparisons of their TLC (Silica gel G. treated with oxalic acid. $CHCl_3$–MeOH (50:3)) and IR spectra (KBr).

Methyl ester of α,β-unsaturated keto-carboxylic acid acetate (XVI). Compound XV (1 g) was dissolved in MeOH and excess of CH_2N_2 in Et_2O was added. After standing overnight in refrigerator. the mixture was treated as usual to give colourless prisms. m.p. 134–135°. $[\alpha]_D^{28}$ −23·4 (*c* = 0·71. $CHCl_3$). yield: 960 mg; IR $\nu_{max}^{CHCl_3}$: no OH; 1755 (OAc). 1695 (C=O); UV λ_{max}^{EtOH}: 243 mμ (log ε 3·87). (Found: C. 52·94; H. 5·92. $C_{27}H_{34}O_{15} \cdot H_2O$ requires: C. 52·59; H. 5·89%).

Catalytic hydrogenation of α,β-unsaturated keto-carboxylic acid acetate (XV). Compound XV (1·85 g) was hydrogenated in EtOH (120 ml) using PtO_2 (300 mg) as catalyst. One mole of H_2 was absorbed to give colourless prisms (XVIII). 1·55 g (from EtOH). m.p. 219–225°. $[\alpha]_D^{28}$ −48·4 (*c* = 0·78. $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH; 1740 (sh) (COOH); 1720 (sh) (C=O). (Found: C. 51·40; H. 6·06. $C_{26}H_{34}O_{15} \cdot H_2O$ requires: C. 51·66; H. 5·96%).

Methylation of XVIII. The hydrogenated product (XVIII) obtained above was methylated with CH_2N_2 as usual. and the product was recrystallized from EtOH to give colourless needles. m.p. 169–170·5°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH; 1765. 1740 (OAc); 1735 (sh) ($COOCH_3$); 1715 (sh) (C=O). (Found: C. 54·10; H. 6·12. $C_{27}H_{36}O_{15}$ requires: C. 54·00; H. 6·04%).

Alkaline hydrolysis of XVIII. A suspension of XVIII (1·7 g) in 2N Na_2CO_3 (30 ml) was heated for 3 hr on a boiling water bath. Deionization with Amberlite IR 120 (H form) followed by evaporation of the solvent gave glassy residue (0·7 g). which was crystallized by treatment with EtOH. Recrystallization from H_2O afforded XIX as colourless prisms (0·4 g), m.p. 180–182°. (Found: C, 46·69; H, 6·73. $C_{16}H_{24}O_{10} \cdot 2H_2O$ requires: C. 46·61; H. 6·84%). The sample free from water of crystallization gave following analytical values. (Found: C. 49·67; H. 6·56. $C_{16}H_{24}O_{10}$ requires: C. 51·06; H. 6·43%); IR ν_{max}^{KBr}: 3600–3400 (OH); 1720. 1710 (sh) (COOH and C=O).

Acid hydrolysis of XIX. Compound XIX (400 mg) was dissolved in 2N H_2SO_4 (15 ml). and heated on a boiling water bath. The reaction product was extracted with Et_2O at short intervals to avoid resinification. The combined ethereal extract was washed with H_2O and dried over Na_2SO_4. Removal of the solvent

gave yellowish syrup. which was chromatographed over silicic acid (15 g) yielding colourless needles of XX (80 mg) from a $CHCl_3$–Me_2CO fraction. These were recrystallized from benzene–n-hexane to give analytical sample. m.p. 134°. $[\alpha] = 0$: IR ν_{max}^{Nujol} cm^{-1}: no OH; 1710 (C=O); 1680 (α,β-unsat. COOH); 1625 (C=C); IR $\nu_{max}^{CHCl_3}$: 3600 (OH). 1787 (w). 1733 (s). 1690 (w) UV λ_{max} 210 mμ (end absorption). 280 mμ (C=O). (Found: C. 61·33: H. 6·09. $C_{10}H_{12}O_4$ requires: C. 61·21; H. 6·17%).

Ozonolysis of XV. Compound XV (0·6 g) was ozonolyzed in CH_2Cl_2 at −50°. After the soln turned deep blue. water containing small amount of AcOH was added and the solvent was evaporated under reduced press. The product was chromatographed over silicic acid (15 g) and $CHCl_3$–MeOH (50:3) fraction gave colourless glassy powder (XXII). which showed a single spot on TLC (silica gel G treated by oxalic acid. CH_2Cl_2–MeOH (50:3): IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH: 3200–2800. 1725. 910 (COOH). 1710 (sh)

(C=O); 1760. 1240 (OAc): NMR: 8·01. 7·96 (5-OAc. CH_3—C(=O)—C): 4·3–3·6 (br. 2H. —COOH. this signal shifts on addition of 1 drop of AcOH). This product showed a positive iodoform reaction.

The same compound was obtained when ozonolysis was carried out in $CHCl_3$ or AcOH. and also when the ozonide was decomposed by H_2O_2 or Zn–AcOH.

Methyl ester of the ozonolysis product (XXII). Methylation of XXII with CH_2N_2 afforded a syrupy product. which was chromatographed over silica gel (20 g). Elution with benzene–Et_2O (8:2) yielded a colourless glassy powder (XXIII) which showed single spot on TLC (silica gel G. benzene–Et_2O (7:3)); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH: 1770–1700 (OAc. COOMe. COMe); UV λ_{max}^{EtOH}: 290 mμ (log ε 1·82). (Found: C. 50·93: H. 5·70. $C_{27}H_{36}O_{17}$ requires: C. 51·26: H. 5·74%).

Baeyer-Villiger oxidation of XXIII. To a soln of 100 mg in $CHCl_3$ (1 ml). the $CHCl_3$ soln of perbenzoic acid[9] (3 ml) and *p*-TsOH (20 mg) was added. The reaction mixture was kept at 25° for 9 days. After dilution with $CHCl_3$. the soln was washed with water and dil. Na_2CO_3. and the $CHCl_3$ layer was dried over Na_2SO_4. The syrupy product was chromatographed over silica gel (15 g). The fractions eluted with benzene gave benzoyl peroxide. From a fraction eluted with benzene–Et_2O (9:1). a small amount of yellowish syrup was obtained. which showed a single spot on TLC (Silica gel G: benzene–Et_2O (1:1)). and was neither benzoyl peroxide nor perbenzoic acid. Its IR spectrum ($CHCl_3$) showed a carbonyl absorption at 1800 cm^{-1} besides acetyl absorption at 1750 cm^{-1}.

Alkaline treatment of paeoniflorin (I). When paeoniflorin I (2 g) was dissolved in 40 ml 2N Na_2CO_3 and heated for 3 hr on a boiling water bath. the soln turned dark brown. Inorganic cations were removed by Amberlite IR 120 (H form) resin and the filtrate was concentrated *in vacuo* until crystals of benzoic acid separated out. Removal of benzoic acid with Et_2O and treatment with active charcoal followed by evaporation of the solvent afforded a syrupy residue which formed crystals from MeOH. Recrystallization from EtOH yielded 400 mg of colourless prisms (XXV). m.p. 216–217°. $[\alpha]_D^{29}$ −50·9 ($c = 0·87$. H_2O). (Found: C. 50·07: H. 6·44. $C_{16}H_{24}O_{10} \cdot \frac{1}{2}H_2O$ requires: C. 49·88: H. 6·54%). The sample free from water of crystallization. m.p. 220–222°. (Found: C. 50·95: H. 6·40; $C_{16}H_{24}O_{10}$ requires: C. 51·06: H. 6·43%); IR ν_{max}^{KBr} cm^{-1}: 3500–3200 (OH): 1750 (lactone).

Alkaline treatment of VI. A soln of VI (130 mg) in 2 ml 2N Na_2CO_3 was heated for 1·5 hr on a boiling water bath and yielded XXV (20 mg) after treating the reaction as described in the experiment using I.

Acetylation of XXV. Acetylation of XXV (100 mg) was carried out using pyridine (1 ml) and Ac_2O (5 ml) at room temp to give 100 mg crude acetate. Recrystallization from EtOH gave colourless needles. m.p. 132–134°. IR spectrum showed no OH absorption: NMR: 8·56 (3H. s. >C—Me): 9·02 (12H. s). 7·95 (3H. s) and 7·89 (3H. s) (6 —OAc): 5·70 (2H. s. —CH_2—OAc). (Found: C. 53·50: H. 5·78. $C_{28}H_{36}O_{16}$ requires: C. 53·50: H. 5·77%).

Product M_1-acetate and product M_2-acetate. A methanolic soln of I (3 g) was added with *p*-TsOH (100 mg) and the soln was refluxed for 17 min on a boiling water bath. The soln was treated with Amberlite IRA 400 (OH form) resin and the solvent was removed *in vacuo* to give a mixture of the products. which showed 3 spots on TLC (Silica gel. Camag Co.. $CHCl_3$–MeOH (7:1). They were tentatively named products M_1. M_2 and M_3 in the order of their R_f values (from the top to the bottom). The mixture was chromatographed over silica gel (20 g) and a mixture of M_1 and M_2 (2·4 g) was obtained from $CHCl_3$–MeOH (95:5) fraction. which was acetylated with Ac_2O and pyridine. Chromatographic separation of acetates afforded crystalline M_1-acetate and M_2-acetate respectively. M_1-acetate was recrystallized from EtOH to give colourless needles. m.p. 123–124°. and was identical with product acetate (IV) by a comparison of their IR spectra (KBr) and mixed fusion. M_2-acetate (0·9 g) was obtained as colourless needles. m.p. 173·5–174·5°: IR ν_{max}^{Nujol}: 1760. 1745 (OAc). 1720 (OBz. ketone): NMR: 8·60 (3H. s) (—C—Me): 8·01 (3H. s). 7·95 (3H. s).

7·94 (6H. s) (4 —OAc): 6·66 (3H. s) (—OMe): 5·49 (2H. s) (—CH_2—OBz): 5·01 (1H. s) (acetal–H): 2·57–1·93 (5H) (—O—Bz). (Found: C. 57·90: H. 5·71. $C_{32}H_{38}O_{15}$ requires: C. 58·0: H. 5·78%).

2.4-*Dinitrophenylhydrazone of product* M_2*-acetate.* Product M_2-acetate gave a mono 2.4-dinitrophenylhydrazone as yellow needles. m.p. 262–265°. (Found: C. 54·30: H. 5·02: N. 6·53. $C_{38}H_{42}N_4O_{18}$ requires: C. 54·16: H. 4·99: N. 6·65%).

K_1-acetate and K_2-acetate. Paeoniflorin (I: 1·7 g) in EtOH was warmed on a water bath at 50–60°. in the presence of 100 mg *p*-TsOH. Treatment of the mixture as described above for M_1 and M_2. gave a mixture of two products named products K_1 and K_2 from a $CHCl_3$ MeOH (9:1) fraction of silica gel chromatography. The mixture was acetylated and the products were separated by silica gel chromatography using benzene–Et_2O for elution to give the respective crystaline acetates. Product K_1-acetate. colourless needles from EtOH. m.p. 164·5–165·5°: IR ν_{max}^{Nujol} cm^{-1}: no OH: 1760 (OAc): 1725 (OBz): 1713 (C=O): NMR: 8·75 (3H. t. J = 6 c/s. —OCH_2CH_3) 8·60 (3H. s) (>C—Me): 7·97 (3H. s). 7·93 (6H. s). 7·88 (3H. s) (4 OAc): 6·23 (2H. q. J = 6 c/s. —OEt). 4·46 (1H. s. acetal–H). 2·5–1·7 (5H. —OBz). (Found: C. 58·62: H. 6·07: $C_{33}H_{40}O_{15}$ requires: C. 58·56. H. 5·96%). K_2-acetate. colourless prisms from EtOH. m.p. 152·5–153·5°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH: 1760. 1745 (OAc); 1725 (sh) (OBz. C=O); NMR: 8·92 (3H. t. J = 7 c/s. —OEt). 8·63 (3H. s) (—C—Me): 8·01 (3H. s). 7·99 (3H. s). 7·97 (3H. s) (4 —OAc); 4·91 (1H. s. acetal–H). 2·7–1·8 (5H. OBz). (Found: C. 58·36: H. 5·73. $C_{38}H_{40}O_{15}$ requires: C. 58·56: H. 5·96%).

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