Tetrahedron Vol. 28, pp. 4309 to 4317. Pergamon Press 1972. Printed in Great Britain

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

THE ABSOLUTE STRUCTURES OF PAEONIFLORIN, ALBIFLORIN, OXYPAEONIFLORIN AND BENZOYLPAEONIFLORIN ISOLATED FROM CHINESE PAEONY ROOT

M. KANEDA, Y. IITAKA and S. SHIBATA*

Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo

(*Received in Japan* 15 *April* 1972; *Received in the UK for publication* 24 *May* 1972)

Abstract– The absolute structure of paeoniflorin, a major principle of Chinese Paeony root (*Paonia albiflora* Pallas (Paeoniaceae)) was established as I and as shown in Chart 2 by the X-ray analysis of a bromo derivative (VI) of product K_2 acetate (V). The absolute structures of the minor constituents, albiflorin (VII), oxypaeoniflorin (IX) and benzoylpaeoniflorin (X) were also established on the basis of the established structure of paeoniflorin.

The absolute structure of paeoniflorin

PREVIOUSLY Shibata *et al.* proposed a structural formula (I) for paeoniflorin, a major chemical constituent of a Chinese drug, "Shaoyao" (in Japanese "Shakuyaku"), the root of *Paeonia albiflora* Pallas (Paeoniaceae).[2] As this structure involving a cage-

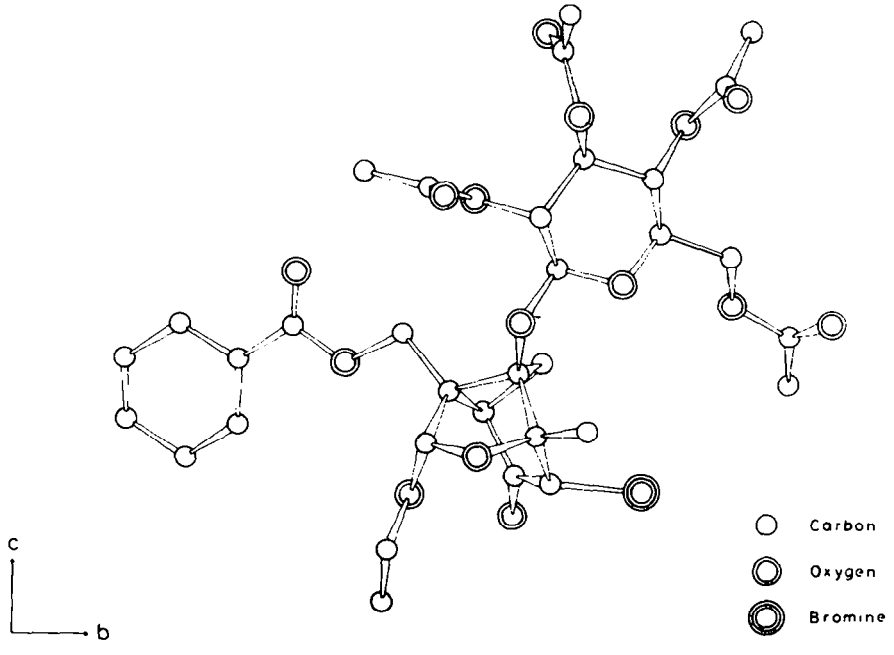
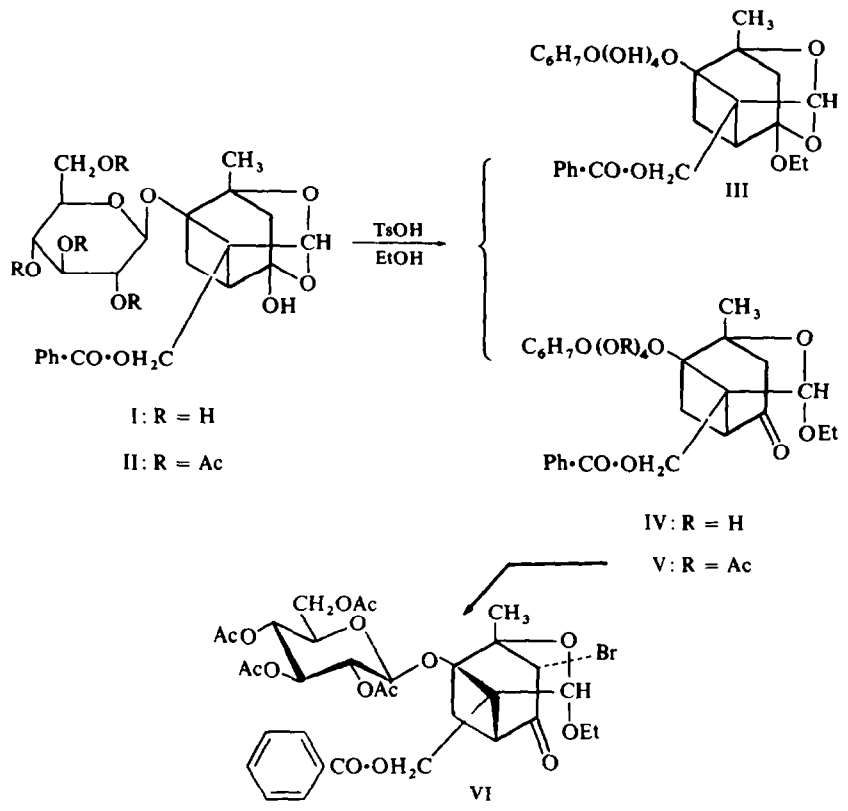


FIG 1.

* Author to whom inquiries should be addressed.

CHART 1



I: R = H

II: R = Ac

like pinane skeleton is very unique among the natural products, an X-ray crystallographic investigation has been undertaken to confirm the structure and establish the absolute configuration.

A bromo derivative (VI) was found to form a single crystal which is suitable for X-ray analysis: On heating paeoniflorin with *p*-toluenesulphonic acid, two products tentatively named K_1 (III) and K_2 (IV)[2] were obtained. The product K_2 acetate (V) was brominanted to afford a monobromo derivative (VI), $C_{33}H_{39}O_{15}Br$, m.p. 199–200°.

The structure was solved by the usual heavy atom method, and its absolute configuration was determined on the basis of anomalous dispersion effect of the Br atom.

CHART 2

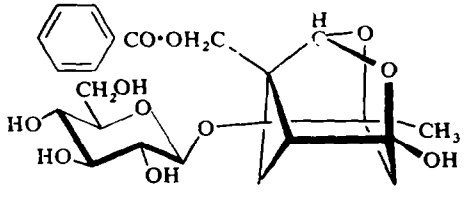


CHART 3

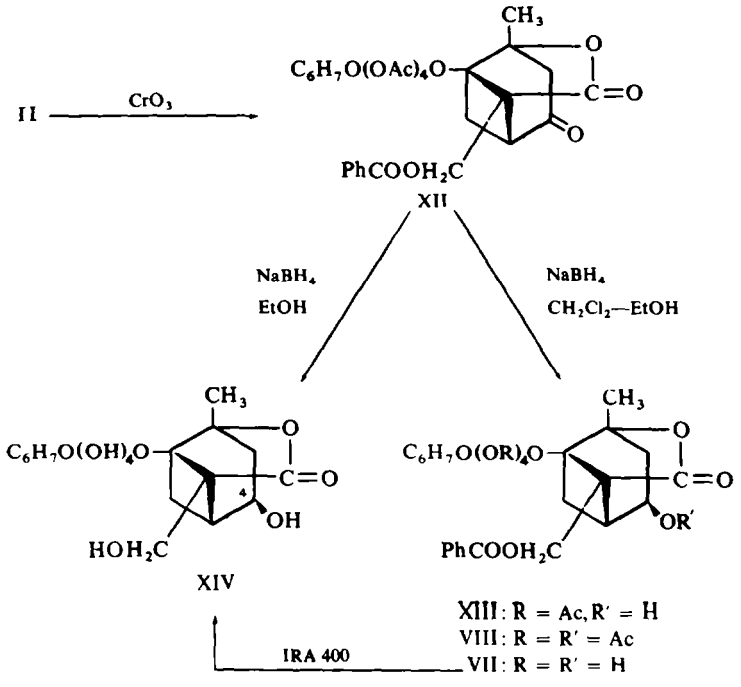
The absolute configuration obtained agreed with that deduced from the fact that the glucosyl group is in the D-type absolute configuration.

The absolute molecular structure of VI determined by X-ray analysis is illustrated in Fig. 1. On the basis of this X-ray analysis and the mechanism of formation of products K_1 (III) and K_2 (IV), the structure proposed for paeoniflorin has been established and is illustrated in Chart 2.

The minor principles of Chinese Paeony root

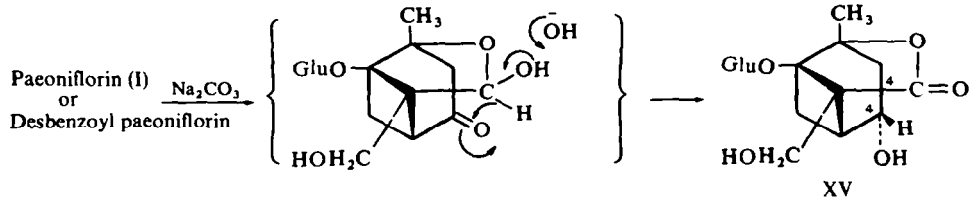
On chromatographical separation of the methanolic extracts of Chinese Paeony root, in addition to paeoniflorin (I), three minor principles named albiflorin (VII), oxypaeoniflorin (IX) and benzoylpaeoniflorin (X) were isolated.

CHART 4



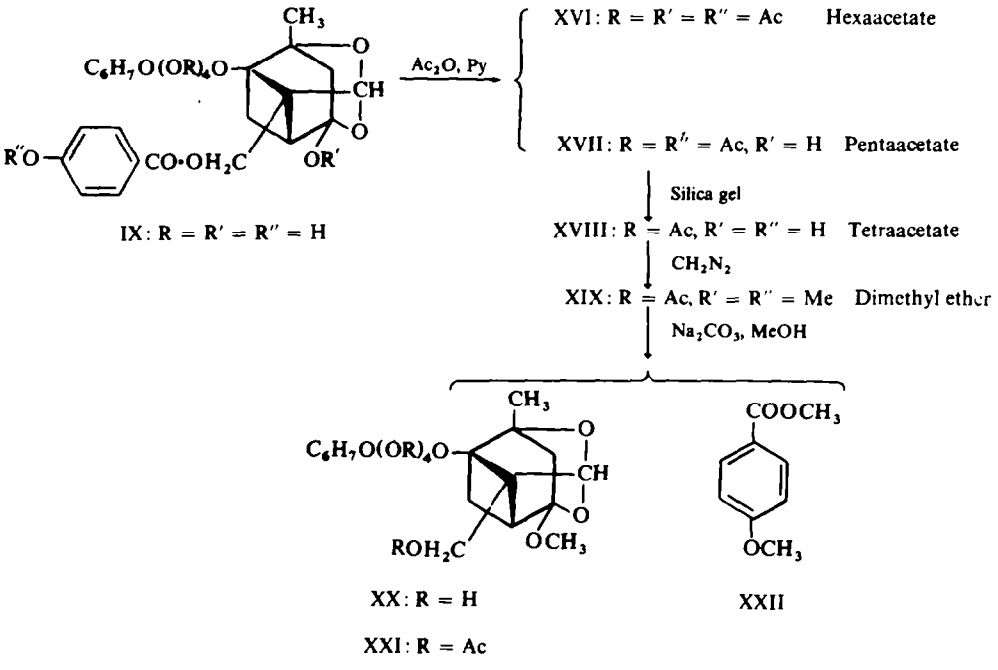
Albiflorin. Albiflorin (VII), an amorphous powder, shows IR absorption of lactonic C=O at 1770 cm^{-1} and an UV absorption of benzoyl at 230 nm; the NMR spectrum gives no signal of an acetal proton and hemiketal OH which are characteristic of paeoniflorin (I). Acetylation of VII afforded a pentaacetate only, which suggested the presence of a readily acetylated OH in the aglycone part.

CHART 5



On the other hand, paeoniflorin tetraacetate (II) was oxidized to afford a keto-lactonic compound (XII) whose ketonic group was reduced with $NaBH_4$ in CH_2Cl_2 and ethanol (1:2) to yield a compound XIII[2]. The acetate of this was identical with albiflorin pentaacetate (VIII). Reduction of XII with $NaBH_4$ in ethanol yielded desbenzoyl albiflorin (XIV) which also was formed from albiflorin by the action of Amberlite IRA-400.

CHART 6



An epimer of XIV at $C_{(4)}$-OH (XV) has been obtained from paeoniflorin (I) or desbenzoyl paeoniflorin by the action of Na_2CO_3. The mechanism of this reaction illustrated in Chart 5, shows that the OH at $C_{(4)}$ must be *trans* to the lactone ring.[3] Thus the OH at $C_{(4)}$ of albiflorin must be *cis* to the lactone ring. The absolute structure of albiflorin has therefore been established as VII.

Oxypaeoniflorin. Oxypaeoniflorin, an amorphous powder, shows UV absorption at 260 nm accounting for a *p*-hydroxybenzoyl grouping. The NMR spectrum has typical A_2B_2 type signals in the aromatic region, and the rest including the acetal and hemiketal signals resemble that given by paeoniflorin. Therefore, this compound is a paeoniflorin homologue having a *p*-hydroxybenzoyl grouping instead of a benzoyl group.

On acetylation oxypaeoniflorin yielded hexa- (XVI) and pentaacetate (XVII) whose NMR spectra show an aromatic acetate signal at δ 2·3 ppm. On passing through a silica gel column the aromatic O-acetyl group of XVII was hydrolysed to give oxypaeoniflorin tetraacetate (XVIII). On methylation oxypaeoniflorin tetra-acetate yielded a dimethyl ether (XIX), which was treated with Na_2CO_3 to afford

methyl anisate (= methyl *p*-methoxybenzoate) (XXII) and desbenzoyl paeoniflorin monomethyl ether (= product F)[2] (XX). Consequently oxypaeoniflorin has the formula IX.

Benzoylpaeoniflorin. Benzoylpaeoniflorin (X), an amorphous powder, shows a benzoyl absorption at 230 nm in the UV spectrum, and its NMR spectrum shows the aromatic proton signals whose intensities are twice stronger than those of paeoniflorin (I) while the rest is almost identical. Thus this compound is probably paeoniflorin monobenzoate. Benzoylation of paeoniflorin afforded a monobenzoate whose triacetate was identical with the triacetate (XI) of the naturally occurring compound. The signal of the methylene at $C_{(6')}$ in the glucosyl moiety appears at δ 4·47 ppm in the NMR spectrum of benzoylpaeoniflorin triacetate, which is 0·25 ppm lower than that given by paeoniflorin tetraacetate (δ 4·22 ppm). This could be due to the difference of the I-effect between benzoyloxy and acetoxyl groupings. It has therefore been concluded that the second benzoyl group of benzoylpaeoniflorin is located at $C_{(6')}$—OH of the glucosyl moiety.

EXPERIMENTAL*

M.ps were determined on a Yanagimoto m.p. apparatus and are uncorrected. NMR spectra were measured on a Japan Electron Optics Lab. JMN-4H-100 (100 MHz) instrument with TMS as the internal standard. Optical rotations were measured with a Yanagimoto Photomagnetic polarimeter Model OR-50. UV absorption spectra were measured in EtOH on a Cary spectrometer Model 11 or a Hitachi ESP-3T. IR spectra were taken on a Japan Spectroscopic Co. DS-402G spectrophotometer. Gas–liquid chromatography was carried out on a Shimadzu Gas Chromatograph Model GC-4A.

The extraction of Chinese Paeony root. The hot methanolic extract of Chinese Paeony root (10 kg) was diluted with water, and washed with ether to remove oily substances. The aqueous layer was exhaustively extracted with BuOH, and the extract was washed several times with acetone. The acetone-soluble fraction was chromatographed on a silica gel column using $CHCl_3$–MeOH (9 : 1) as the eluting solvent to separate successively benzoylpaeoniflorin (*ca* 1 g), paeoniflorin (*ca* 100 g), albiflorin (*ca* 10 g) and oxypaeoniflorin (*ca* 6 g).

Bromo-compound (VI) *derived from product* K_2 (IV). K_2 acetate (V) (1·3 g),[2] was dissolved in $CHCl_3$ (50 ml) and cooled with ice–water. To this soln, Br_2 (2 ml) was added dropwise, and the mixture allowed to stand overnight at room temp. After evaporation of the solvent under a reduced pressure at 50°, the residue was dissolved in $CHCl_3$ and the solvent was evaporated again *in vacuo*. This procedure was repeated several times until the red colour of Br_2 disappeared. Then the residue was finally dissolved in $CHCl_3$, and the soln washed with water and dried over Na_2SO_4. Evaporation of the solvent left an almost colourless syrup, which was chromatographed on a silica gel column eluting with 5% acetone in benzene. The fractions of the major product were combined and evaporated to give a colourless syrup, from which the crystals of bromo compound VI (300 mg), m.p. 199–200°, were obtained on recrystallization from MeOH. $[\alpha]_D^{18}$ −7·3° (c = 1·4, $CHCl_3$): λ_{max}^{EtOH} 228, 274, 280 nm (log ε, 4·16, 3·02, 2·95): ν_{max}^{KBr} 1770–1710 (broad, OAc, OBz, ketone) cm^{-1}: NMR (in $CDCl_3$) δ 1·10 (3H, t, J = 7 Hz, CH_3—CH_2—O—), 1·65 (3H, s, $\diagdown C(Br)(H)\diagup$—$CH_3$), 1·99, 2·02, 2·05, 2·07 (1 Me each, s, 4 OAc), 4·14 (2H, d, J = 5 Hz, C_6·—H_2), 4·40 (1H, s, $\diagdown C(Br)(H)\diagup$), 4·47 (2H, s, —$CH_2$—OBz), 5·10 (1H, s, acetal H), 7·42–8·03 (5H, Ar—H) ppm. (Found: C, 52·45: H, 5·40: Br, 10·63. $C_{33}H_{39}O_{15}Br$ requires: C, 52·49: H, 5·21: Br, 10·60%).

* The full details and the experiments of the X-ray crystallographic studies of the bromo-compound (VI) is described in a paper submitted to *Acta Cryst.* B **28** (1972) in press.

Albiflorin (VII). Colourless hygroscopic powder: $[\alpha]_D^{22}$ −19·9° (*c* = 0·95, EtOH): λ_{max}^{EtOH} 231, 267 (sh), 274, 281 nm (log ε, 4·05, 3·15, 3·16, 3·08): ν_{max}^{KBr} 3400 (broad, OH), 1760 (lactone), 1720 (OBz), 1605, 1590 (phenyl) cm^{-1}; NMR (in $(CD_3)_2CO$) δ 1·46 (1 Me, $-\underset{}{\overset{}{C}}-CH_3$), 7·48–8·15 (5H, Ar—H) ppm.

Albiflorin pentaacetate (VIII). Albiflorin VII (100 mg), pyridine (1 ml) and Ac_2O (1 ml) were mixed under ice-cooling, and the mixture was allowed to stand overnight in a refrigerator. After working up in the usual way, the crude product was chromatographed over silica gel. Elution with benzene–acetone (10:1) and evaporation of the solvent yielded a colourless powder of albiflorin pentaacetate (VIII), which showed a single spot on TLC, but failed to crystallize; NMR (in $CDCl_3$) δ 1·48 (1 Me, $-\underset{}{\overset{}{C}}-Me$), 1·91, 1·95, 2·00, 2·05 (1 Me each, 4-OAc), 2·15–3·25 (5H, 2 $\diagdown CH_2$, $-\underset{}{\overset{}{C}}-H$), 3·57 (1H, m, C_5·—H), 4·10 (2H, br.d, C_6·—H_2), 4·57 (2H, ABq, *J* = 12 Hz, $-CH_2-OBz$), 4·71–5·20 (5H, C_1·~C_4·—H, $\diagdown\overset{OH}{\underset{H}{C}}\diagup$), 7·38–8·05 (5H, Ar–H) ppm.

Acetylation of the reduction product (XIII) *of the keto-lactone* (XII). XII (15 mg) in pyridine (0·5 ml) and Ac_2O (0·5 ml) was allowed to stand overnight at room temp. Usual treatment afforded a colourless powder (8 mg), which showed a single spot on TLC but failed to be obtained in a crystalline form. Albiflorin pentaacetate (VIII) was proved to be identical with this product by comparison of TLC and IR spectra (in KBr).

Debenzoylation of albiflorin (VII). To a soln of albiflorin (100 mg) in H_2O (2 ml), Amberlite IRA 400 resin (OH form) (2 ml) was added. The mixture was stirred for 24 hr at room temp, filtered from the resin and the solvent was evaporated *in vacuo*. Treatment of the residual syrup with MeOH gave a colourless powder, which was recrystallized from MeOH to yield colourless plates of XIV (31 mg), m.p. 241–243°, $[\alpha]_D^{23}$ −33·3° (*c* = 0·96, H_2O), which was identical with the reduction product (XIV) obtained from XII by the action of $NaBH_4$ in EtOH,[2] m.p. 239–242°, $[\alpha]_D^{26}$ −28·9° (*c* = 0·83, H_2O), by mixed fusion and comparison of TLC and IR spectra.

Oxypaeoniflorin (IX). A colourless, hydroscopic powder, $[\alpha]_D^{22}$ −13·0° (*c* = 0·92, EtOH); λ_{max}^{EtOH} 260, 275 (sh.) nm (log ε, 4·12, 3·94); ν_{max}^{KBr} 3400 (broad, OH), 1710 (*p*-OHC_6H_4CO—), 1610, 1595 (phenyl) cm^{-1}; NMR (in $(CD_3)_2CO$) δ 1·33 (1 Me, $-\underset{}{\overset{}{C}}-Me$), 5·38 (1H, s, acetal-H), 6·10, (1H, br..s, hemiketal OH), 6·91, 7·90 (a pair of doublets, 2H each, *J* = 8 Hz, *p*-OH—C_6H_4—CO—) ppm.

Acetylation of oxypaeoniflorin (IX). Oxypaeoniflorin IX (200 mg), pyridine (2 ml) and Ac_2O (15 ml) were mixed under ice-cooling, and the mixture was allowed to stand overnight in a refrigerator. The crude products were examined by TLC in comparison with paeoniflorin tetra- and pentaacetate and oxypaeoniflorin pentaacetate seemed to exist as the main product along with a small amount of hexaacetate. The mixture was chromatographed over silica gel (Wakogel 70 g) using benzene–acetone (10:1) as the eluting solvent.

On evaporation of the first fraction, a colourless syrup of XVI was obtained, which showed a single spot on TLC, but failed to crystallize (35 mg): NMR (in $CDCl_3$) δ 1·33 (1 Me, $-\underset{}{\overset{}{C}}-Me$), 1·97 (1 Me), 2·02, 2·09 (2 Me, each) (5 × OAc), 2·30 (1 Me, $OCOC_6H_4$—OAc), 3·65 (1H, m, C_5—H), 4·13 (2H, br. d, *J* = 4 Hz, C_6—H_2), 4·51 (2H, AB q, *J* = 11 Hz, $-CH_2-OCOC_6H_4$—OAc), 5·49 (1H, s, acetal-H), 7·20, 8·07 (a pair of doublets, 2H, each, *J* = 9 Hz, $-OCOC_6H_4$—p(OH)) ppm.

The second fraction afforded a small amount of a colourless solid, which was recrystallized from benzene to yield colourless needles of XVII (a few mg), m.p. 181–183°; $\nu_{max}^{CHCl_3}$ 1760, 1725 (sh), 1608 cm^{-1}.

The third fraction gave a colourless syrup, which was crystallized from acetone as colourless needles of XVIII (93 mg), m.p. 240–241°; ν_{max}^{KBr} 1745 (OAc), 1715 (—$OCOC_6H_4$—OH), 1613, 1592 (phenyl); NMR (in $(CD_3)_2CO$) δ 1·29 (1 Me, $-\underset{}{\overset{}{C}}-Me$), 3·90 (1H, m, C_5·—H), 4·14 (2H, d, *J* = 4 Hz. C_6·—H_2), 4·46 (2H, ABq, *J* = 11 Hz, $-CH_2-OCOC_6H_4$—OH), 5·31 (1H, s, acetal-H), 6·03 (1H, br. s, hemiketal OH), 6·89, 7·90 (2H each, a pair of doublets, *J* = 9 Hz, $OCOC_6H_4$—p(OH)) ppm. (Found: C, 55·84; H, 5·52. $C_{31}H_{36}O_{16}$ requires: C, 56·07; H, 5·47%).

When the acetate mixture was chromatographed on a column of silica gel "Kantokagaku", a larger amount of XVII was obtained than above (1 g of IX yielded 260 mg of pentaacetate, along with 80 mg of

hexaacetate and 120 mg of tetraacetate). NMR (in $(CD_3)_2CO$) δ 1·27 (1 Me, $-\underset{\diagup}{\overset{\diagdown}{C}}-$Me), 2·24 (1 Me, Ar—OAc), 3·90 (1H, m, C_5·—H), 4·12 (2H, d, J = 4 Hz, C_6·—H_2), 4·49 (2H, ABq, J = 12 Hz, $-CH_2-OCOC_6H_4-$p(OH)), 5·33 (1H, s, acetal-H), 6·08 (1H, s, hemiketal-OH), 7·20, 8·01 (a pair of doublets, 2H each, J = 8 Hz, $-OCOC_6H_4-$p(OH)) ppm. (Found: C, 55·62; H, 5·31. $C_{33}H_{38}O_{17}$ requires: C, 56·14; H, 5·43%).

Dimethyl ether of oxypaeoniflorin tetraacetate (XIX). To a soln of XVIII (90 mg) in MeOH, excess of CH_2N_2 in Et_2O was gradually added at 0°. After standing at room temp for 3 days, the solvent was removed and the residual syrup was chromatographed over silica gel in benzene–acetone (10:1) to afford a colourless syrup, which was treated with water to solidify as crystalline XIX (31 mg); NMR (in $CDCl_3$) δ 1·34 (1 Me, $-\underset{\diagup}{\overset{\diagdown}{C}}-$Me), 1·96, 2·00, 2·02, 2·05 (1 Me each, 4 × OAc), 3·39, 3·85 (1 Me each, 2 × OMe), 4·13 (2H, br. d, C_6-H_2), 4·47 (2H, ABq, J = 12 Hz, $-CH_2-OCOC_6H_4-$p(OH)), 5·42 (1H, s, acetal-H), 6·92, 7·96 (a pair of doublets, 2H each, J = 9 Hz) ppm.

Alkaline hydrolysis of XIX. XIX (30 mg) was dissolved in MeOH (2 ml) and 2N Na_2CO_3 (2 ml), and the soln was warmed on a boiling water bath for 3 hr. The mixture was diluted with water and extracted with $CHCl_3$. The chloroform soln was washed with water, dried over Na_2SO_4, concentrated and detected with GLC: column: PEG 20M on Chromosorb W; column temp, 180°; carrier gas: N_2 (1 Kg/cm^2). The presence of methyl *p*-methoxybenzoate (methyl anisate) (XXII) in the residue was proved (retention time: 8·3 min) in comparison with the authentic sample.

The aqueous layer was neutralized with Amberlite IR 120 (H form) resin, and the solvent was evaporated to yield colourless syrup which gave a single spot and the same R_f value as product F (XX) on TLC. This product was acetylated with Ac_2O and pyridine, and crystallization of the acetate from aqueous MeOH afforded colourless needles (12 mg), m.p. 130–131°, which were identical with product F acetate (XXI) by a mixed fusion and comparisons of their TLC and IR spectra (KBr).

Benzoylpaeoniflorin (X). A colourless, glassy powder, λ_{max}^{EtOH} 230, 267 (sh.), 274, 281 nm (log ε, 4·38, 3·18, 3·26, 3·17); ν_{max}^{KBr} 3400 (br., OH), 1725–1700 (OBz), 1605, 1588 (phenyl) cm^{-1}; NMR (in $(CD_3)_2CO$) δ 1·25 (1 Me, $-\underset{\diagup}{\overset{\diagdown}{C}}-$Me), 5·38 (1H, s, acetal-H), 6·12 (1H, s, hemiketal-OH), 7·41–8·13 (10H, 2 × OBz) ppm.

Benzoylpaeoniflorin triacetate (XI). Benzoylpaeoniflorin X (120 mg) was acetylated with Ac_2O (2 ml) and pyridine (2 ml) at 0°. The product showed two spots on TLC, and the main product having smaller R_f value was collected by column chromatography over silica gel in benzene–acetone (7:1), and crystallized from aqueous acetone to yield colourless needles (35 mg), m.p. 151·5–154°, $[\alpha]_D^{20}$ 27·4° (c = 0·84, EtOH); ν_{max}^{KBr} 3400 (OH), 1750 (OAc), 1725 (OBz), 1605, 1586 (phenyl) cm^{-1}; NMR (in $(CD_3)_2CO$) δ 1·27 (1 Me, $-\underset{\diagup}{\overset{\diagdown}{C}}-$Me), 4·14 (1H, m, C_5·—H), 4·47 (2H, d, J = 5 Hz, C_6·—H_2), 4·56 (2H, ABq, J = 12 Hz, CH_2—OBz), 5·37 (1H, s, acetal-H), 6·05 (1H, s, hemiketal OH), 7·45–8·15 (10H, 2 × OBz) ppm. (Found: C, 60·83; H, 5·40. $C_{36}H_{38}O_{15}$ requires: C, 60·90; H, 5·39%).

Benzoylation of paeoniflorin (I). To a soln of I (1 g) in pyridine (1·5 ml), benzoyl chloride (360 mg) was added under ice cooling. After standing 1 hr at 0°, the mixture was poured into ice water, extracted with $CHCl_3$ and evaporated to dryness. The residue was submitted to chromatography on a silica gel column using benzene–acetone (7:3) as eluting solvent to give colourless syrup of paeoniflorin monobenzoate which showed a single spot and the same R_f value on TLC as X. From the fractions which were eluted preceding to the monobenzoate on the column chromatography, a small amount of highly benzoylated products were also obtained.

Paeoniflorin monobenzoate triacetate (XI). Paeoniflorin monobenzoate (200 mg), Ac_2O (2 ml) and pyridine (2 ml) were mixed under ice cooling and allowed to stand 4 hr at 0°. The main product obtained by chromatographic separation of the mixture, was recrystallized from aqueous acetone to afford colourless needles (137 mg), m.p. 153–154°, and found to be identical with XI by a mixed fusion and comparisons of IR spectra (KBr) and TLC.

Acknowledgements—We are indebted to the members of the microchemical and spectroscopical laboratories of this Faculty for microanalyses and UV, IR, and NMR spectral measurements. We thank the Mitsubishi Foundation for the financial support.

REFERENCES

[1] Part XXXII. T. Akiyama, O. Tanaka and S. Shibata, *Chem. Pharm. Bull. Tokyo* **20** (1972) in press
[2] N. Aimi, M. Inaba, M. Watanabe and S. Shibata: *Tetrahedron* **25**, 1825 (1969), and references therein
[3] D. Dvornik and O. E. Edwards: *Proc. Chem. Soc.* 280 (1958); R. Hayashi: *Chem. Pharm. Bull.* **15**, 139 (1967)