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THE STRUCTURE OF BOSTRYCIN

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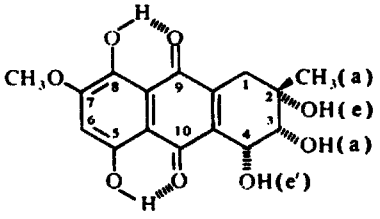
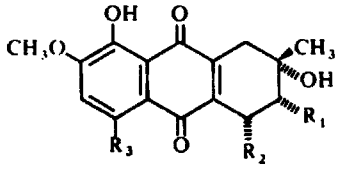
Abstract—Bostrycin, a novel tetrahydroanthraquinone pigment[1] has been isolated from cultures of *Bostryconema alpestre* cesati.

Structure I is proposed for this pigment based on chemical and spectroscopic evidence[2] and X-ray diffraction study.[3]

BOSTRYCIN I*, a new red pigment antibiotic which is effective against mainly gram positive bacteria, was extractable with chloroform from the broth culture of *Bostryconema alpestre* cesati. After purification by recrystallization (pyridine–water), it melted at 222–224° (dec) and was poorly soluble in all common organic solvents.





II: $R_1 = R_2 = R_3 = OAc$

III: $R_1 = R_3 = OH$, $R_2 = H$

Bostrycin, I, $C_{16}H_{16}O_8$ (M.W. 336·29), M^+ ion *m/e* 336, exhibited IR (3360, 1595 cm^{-1}) and UV spectra (λ_{max} 472, 505, 542 mμ) characteristic for the naphthazarin-type nucleus (5,8-dihydroxynaphthoquinone)[4] (Fig. I). This supported by the NMR spectrum of bostrycin I (Fig. II) which shows two H bonded OH groups at $\delta = 12{\cdot}42$ and 13·12 as broad singlets which are exchanged with deuterium oxide.[5]

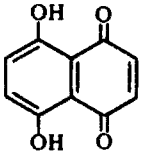
Furthermore the NMR spectrum shows three broad OH groups ($\delta = 4{\cdot}45$, 4·70, and 5·16), one OMe group ($\delta = 3{\cdot}90$, 3H, s) and one aromatic proton ($\delta = 6{\cdot}41$, 1H, s). After being shaken with a little D_2O, the broad peaks of three OH groups disappear and the multiplet for one proton at $\delta = 4{\cdot}73$ (C-4) appears as doublet ($J = 4{\cdot}5$) coupling with C-3 proton at $\delta = 3{\cdot}51$ (d, $J = 4{\cdot}5$) in an AB system. The C-3 and C-4 protons ($\delta = 3{\cdot}51$ and 4·73 in I) show a pair of lower field doublets at $\delta = 5{\cdot}02$ and 5·93 ($J = 6{\cdot}0$) in the triacetate (II) which was obtained by acetylation with acetic anhydride in pyridine. The Me region exhibits only one unsplit 3-proton signal at $\delta = 1{\cdot}23$ in I and was little effected by acetylation ($\delta = 1{\cdot}29$ in II). The low δ-value suggests that the Me is attached to quaternary C atom bearing at least one O atom. Summarizing the information obtained, the partial structure (A) was established.

A 2-proton "singlet" at $\delta = 2{\cdot}68$ in I exhibits an isolated allylic methylene protons.[6]

The triacetate II; $C_{22}H_{22}O_{11}$, m.p. 255·5–260·5°, shows IR bands at 3400, 1637 and 1602 cm^{-1}, confirming the presence of the H-bonded OH free quinone CO and H-bonded quinone CO groups respectively. These are in accorded with the UV

* Symbols a, e, and e' denote the axial, equatorial and pseudo equatorial configuration.

(Fig. I) and NMR spectra which show an H-bonded OH proton (δ = 12·39, 1H, broad s) a tertiary OH proton (δ = 4·95), two alcoholic acetoxyl groups (δ = 2·00, 6H, s) and one phenolic acetoxyl group (δ = 2·16, 3H, s). Consequently, the structure II was proposed for the triacetate.



—C—(OH)—CH(OH)—C(CH$_3$)(OH)—
one aromatic proton
one aromatic OMe group
partial structure (A)

Bostrycin I consumed about two moles of H_2 (10% Pd-C in pyridine) to give a greenish brown compound which was immediately oxidized by air into red desoxybostrycin III.

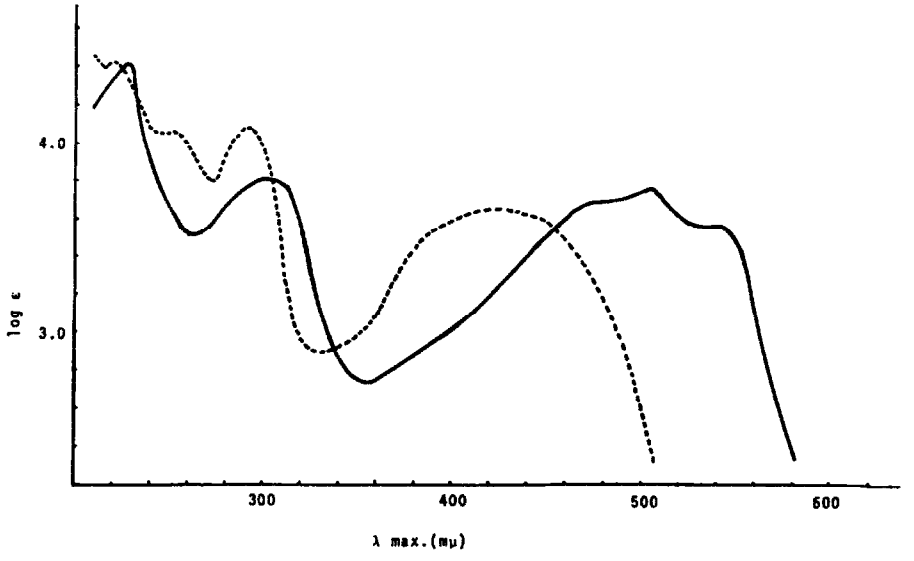


FIG. 1. Absorption spectra in ethanol of bostrycin I(—) and its triacetate II (. . . .).

Desoxybostrycin III has the molecular formula $C_{16}H_{16}O_7$, according to the analytical data and mol wt (320) deduced from it's mass spectrum. The IR and UV spectra are similar to those of bostrycin. These data show that the benzylic OH group in I was eliminated by hydrogenolysis. In the NMR spectrum of III, the four methylene protons at the C-1 and C-4 are observed at 2·62–2·80 ppm as broad peaks, and the C-4 OH proton in I has disappeared.

The unstable greenish brown compound in pyridine, which could not be isolated, was acetylated with acetic anhydride in the absence of air to give leuco-penta-acetate IV (amorphous) and a small amount of crystalline product V.

The NMR spectrum of the penta-acetate IV (Fig. II) was in accordance with the structure IV. The C-3 proton appeared as a triplet at δ 5·03 (J = 6·0) coupling with C-4 methylene protons (δ 2·50–2·91) which, overlapping with the C-2 methylene protons, was deshielded by acetoxy CO groups. Four equivalent acetoxyl groups (C-5,

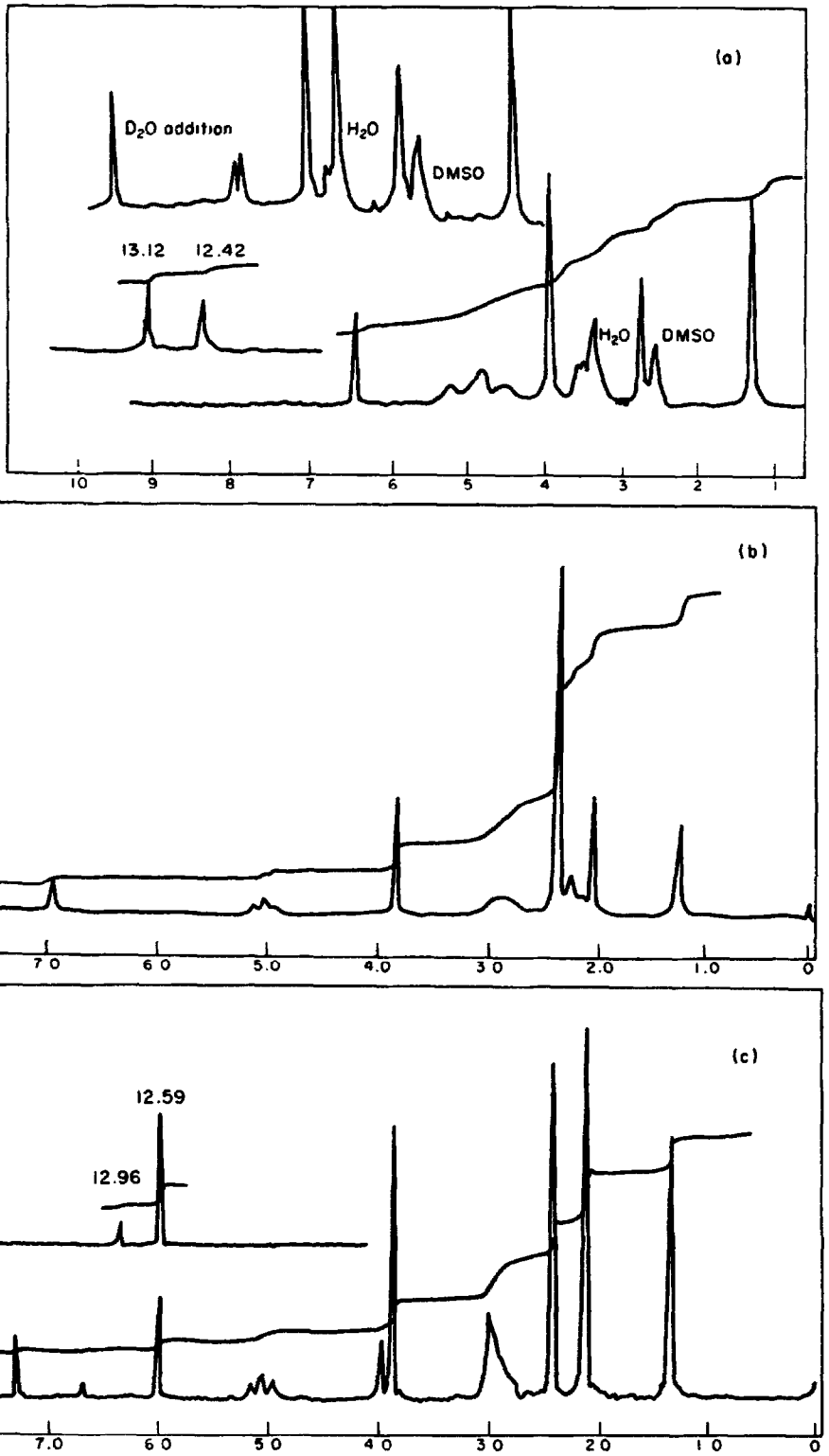
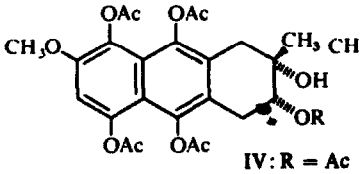


FIG. 2. NMR spectra of (a) bostrycin I in DMSO, (b) penta-acetate IV in $CDCl_3$ and (c) diacetate VI in $CDCl_3$.

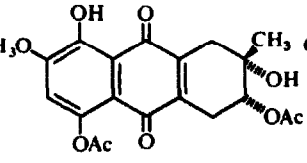
C-8, C-9 and C-10 OAc) and one alcoholic acetoxyl group (C-3-OAc) appear as singlets at δ 2·36 and 2·05 ppm respectively.

The minor white crystalline product V, $C_{24}H_{26}O_{11}$, m.p. 205–207, exhibited a UV spectrum similar to the compound IV, but in the IR spectrum the CO absorption at 1745 cm^{-1}, which was seen in IV as a shoulder, had disappeared. The C-3 OH proton appeared as doublet at δ 4·77 (J = 5) coupling with the C-3 proton which was seen at δ 3·61 as a multiplet.

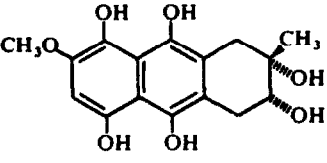
From the above results, the unstable greenish brown compound in pyridine must be have the structure IIIa.



IV: R = Ac
V: R = H

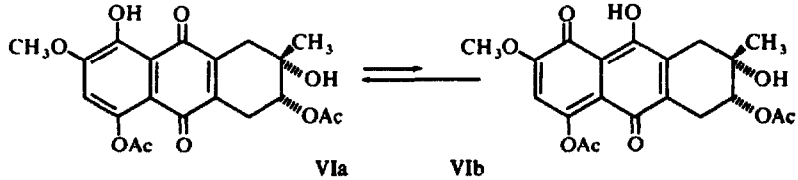


VI



IIIa

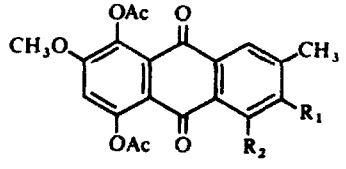
When desoxybostrycin was treated with acetic anhydride in pyridine it gave diacetate VI, $C_{20}H_{20}O_9$, m.p. 206–209°, which was also obtained by hydrogenolysis of the triacetate II in chloroform with Pd-C (10%). In the IR spectrum, the absorptions at 3460 and 1712 cm^{-1} in KBr disk are shown at 3670, 3580–3400 and 1720 (sh) cm^{-1} in chloroform solution. From these observations, the compound VI must have a OH group intramolecularly H-bonded with an acetoxyl group. In the NMR spectrum (Fig. II), each of the two OMe peaks (δ 3·89, 3·96), C-6 proton (δ 5·98, 6·68) and the C-8 proton (δ 12·59, 12·96) seemed to be in tautomerism as follow.



VIa VIb

As the intensities of the latter peaks are less than 10%, the molecules of VI must predominantly exist in the form of VIa in chloroform solution.

Bostrycin I was readily dehydrated by refluxing it in formic acid to yield the anthraquinone derivatives, which were treated with acetic anhydride in pyridine to give three acetates: triacetate VII (main product), diacetate (VIII) and tetracetate IX (minor product). The IR and NMR data are shown in Table 1.



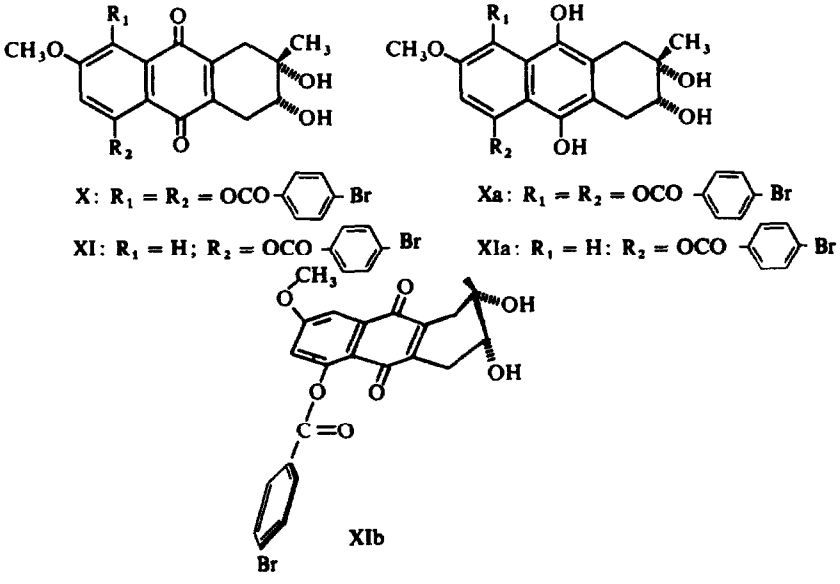
VII: R_1 = OAc; R_2 = H
VIII: R_1 = R_2 = H
IX: R_1 = R_2 = OAc

In the NMR spectra, the doublet of the C-3 proton in I (3·15 ppm J = 4·5 c/s, after D_2O addition) and triacetate II (5·02 ppm, J = 6·0 c/s) appear as the triplet in

TABLE 1. NMR SPECTRA OF ANTHRAQUINONE DERIVATIVES

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
VII	8·03	2·30	2·34	7·81	2·46	6·92	3·93	2·46
VIII	7·94, d, J = 2·0	2·47	7·94, q, J = 7·6, 2·0	8·05, d, J = 7·6	2·47	6·91	3·93	2·47
IX	7·98	2·31	2·34	2·46	2·40	6·89	3·90	2·40

diacetate VI (5·05 ppm, J = 6·0 c/s) and leucopenta acetate IV (5·03 ppm, J = 6·0 c/s). According to these data, the C-3 proton is shown to have the equatorial and the C-3 OH group the axial configuration. The chemical shifts of the C-2 Me (1·23 and 1·18 ppm, for I and III respectively) are not effected by the C-3 and C-4 acetoxyl groups (1·29 and 1·25 ppm for II and VI respectively). These data show that the C-2 Me must be axial and the C-4 OH a puseudo-equatorial configuration. If the C-4 OH group and C-2 Me group has a 1,3-diaxial relationship in I, the C-2 Me signal in the triacetate II would appear down-field by about 0·2 ppm from the position of the corresponding signal in I.[7] These are all supported by the X-ray diffraction study of the *p*-bromozoate derivative (XI).



When bostrycin was treated with *p*-bromobenzoylchloride in pyridine, it did not give a crystalline product, but IIIa which had very activated OH groups, gave an amorphous unstable compound X and yellow material XI,* which was crystallized from ethanol as fine needles. Although compounds X and XI were separated by silica-gel column chromatography, X was decomposed gradually in methanol solution.

Compound X was further hydrogenated with 10% Pd-C to give *p*-bromobenzoic acid and the yellow material which was identical with XI in all respects. From these observations and the result of the acetylation of I to IV and V, it seemed that IIIa was

* We received a private communication from Dr. A. Stoessl that the hydrolysis product of XI was identical with altersolanol B by TLC and in its UV spectrum.

benzoylated to dibenzoate Xa and oxized by air to X or further hydrogenated to XIa. XI was obtained by air oxidation of XIa.

When compound XI was crystallized from DMF, it yielded single crystals suitable for X-ray analysis containing one mole of DMF. The molecular structure of XI was found to be XIb from the three dimensional X-ray diffraction study. The relative positions of the Me and the OMe groups were now assigned the C-2 and C-7 positions respectively in accordance with many naturally occurring anthraquinones.[9]

These chemical and physicochemical data show that bostrycin has the structure I.

EXPERIMENTAL

Isolation and properties of bostrycin I. A strain of *Bostryconema alpestre* cetati was cultured in a medium (pH 6·0) containing 3% potato extract, 2% glucose 0·5% pharma-media, 0·5% KH_2PO_4 and 0·25% $MgSO_4$, which was one of the suitable media for production of bostrycin, at 26° for 5 days. The cultured broth (20 l.) was extracted with $CHCl_3$ (60 l.) 5 times to give 25 g of the crude antibiotic which was crystallized from pyridine–water; m.p. 222–224° (dec). (Found: C, 57·02; H, 4·79. $C_{16}H_{16}O_8$ requires: C, 57·14; H, 4·80; M^+ ion *m/e* 336; IR (KBr) 3510, 3480, 3360, 1595 cm^{-1}; λ_{max}^{EtOH} 228 (2·67 × 10^4), 303 (6·9 × 10^3), 472 (5·10 × 10^3), 505 (6·00 × 10^3), 542 mμ (ε 3·90 × 10^3), δ_{ppm}^{DMSO}: see Fig. II.

Triacetate (II)

Bostrycin I (5·0 g) in 20 ml pyridine was treated with 21 ml Ac_2O and kept overnight at room temp. The mixture was then poured into ice–water (700 ml) to give 3·5 g of the crude acetate. By means of silica gel chromatography and elution with chloroform-acetone (4:1), 2·9 g of II was obtained, which was crystallized from acetone–water; m.p. 255·5–260·5°. (Found: C, 57·17; H, 4·81. $C_{22}H_{22}O_{11}$ requires: C, 57·14; H, 4·80%); IR (KBr) 3400, 1772 (sh), 1743, 1722 (sh), 1637, 1602; δ_{ppm}^{DMSO}: 1·29 (3H, s, C-2-CH_3), 2·01 (6H, s, C-3, C-4-OAc), 2·18(3H, s, C-5-OAc), 2·90 (C-1-CH_2), 3·77 (3H, s, C-7-OCH_3), 4·95 (1H, C-2-OH), 5·02 (1H, d, J = 6 cs, C-3-H), 5·97 (1H, d, J = 6 cs, C-4-H), 6·08 (1H, s, C-6-H), 12·39 (1H, s, C-8-H).

Desoxybostrycin III

Bostrycin I (1 g) absorbed about 2 moles H_2 upon hydrogenation over 5% pd-C (1·2 g) in 70 ml pyridine. The catalyst was filtered off, the filtrate was concentrated about to 10 ml *in vacuo* and added about 60 ml light petroleum to give crude desoxybostrycin (653 mg), which was crystallized from pyridine–ether. (Found: C, 60·01; H, 4·99. $C_{16}H_{16}O_7$ requires: C, 60·00; H, 5·04%); M^+ ion *m/e* 320; IR (KBr): 3450 (sh), 3400, 1603 cm^{-1}; λ_{max}^{EtOH}: 234 (3·14 × 10^4), 305 (8·45 × 10^3), 478 (6·59 × 10^3), 505 (7·24 × 10^3), 542 mμ (ε = 4·42 × 10^3); δ_{ppm}^{DMSO}: 1·18 (3H, s, C-2-CH_3), 2·62–2·80 (4H, m, C-1-CH_2, C-4-CH_2), 3·55 (1H, m, C-3-H), 3·88 (3H, s, C-7-OMe), 4·22 (1H, broad s, C-2-OH), 4·72 (1H, broad s, C-3-OH), 6·38 (1H, s, C-6-H), 12·37 (1H/s, C-8-OH), 12·90 (1H, s, C-5-OH).

Leucopenta-acetate (IV) *and tetra-acetate* (V)

Bostrycin I (1 g) was dissolved in 70 ml pyridine and 5% pd-C (1 g) added. H_2 gas bubbled through with stirring. After about 1 hr the red color of the soln turned to the greenish brown, at this time 4·2 ml Ac_2O was added with passage of H_2 gas for 4 hr in the absence of air. After filtration of the catalyst, the filtrate was concentrated about to 20 ml and poured into 200 ml ice–water to give 1·164 g of crude acetates. From chromatography of the crude acetates on silica gel (50 g), 423 mg of IV was eluted with benzene/EtOAc (1:1). (Found: C, 58·68; H, 5·50. $C_{26}H_{28}O_{12}$ requires: C, 58·64; H, 5·30%); IR (KBr): 3480, 1778 (sh), 1767, 1745, 1720 (sh) cm^{-1}; λ_{max}^{EtOH}: 233 (4·2 × 10^4), 282·5 (4·9 × 10^3), 295·5 (6·2 × 10^3), 303 (5·8 × 10^3), 335 (3·0 × 10^3), 347 mμ (ε 3·3 × 10^3); $\delta_{ppm}^{CDCl_3}$ 1·25 (3H, s, C-2-CH_3), 2·05 (3H, s, C-3-OAc), 2·24 (1H, s, C-2-OH), 2·05 (3H, s, C-3-OAc), 2·24 (1H, s, C-2-OH), 2·36 (12H, C-5, C-8, C-9, C-10-OAc), 2·68–3·16 (4H, m, C-1-CH_2, C-4-CH_2), 3·84 (3H, s, C-7-OMe), 5·03 (1H, t, J = 6·0 cs, C-3-H), 6·94 (1H, s, C-6-H). Tetra-acetate V was obtained in the polar fractions eluted with bezene/EtOAc (1:2), which was crystallized from EtOAc to give 103 mg fine needles; m.p. 205–207°. (Found: C, 58·40; H, 5·39; $C_{24}H_{26}O_{11}$ requires: C, 58·77; H, 5·34%); $[\alpha]_D^{22}$ −21·0° (c, 1·0, in pyridine); IR (KBr): 3300 (broad), 1760 cm^{-1}; λ_{max}^{EtOH} 239 (9·3 × 10^4), 284 (4·9 × 10^3); δ_{ppm}^{DMSO} 1·15 (3H, s, C-2-Me), 2·3–2·5 (12H, C-5, C-8, C-9, C-10-OAc), 2·58–2·92 (4H, C-1-CH_2, C-4-CH_2), 3·61 (1H, m, C-3-H), 4·43 (1H, C-2-OH), 4·77 (1H, d, J = 5 cs, C-3-OH), 7·37 (1H, s, C-6-H).

Diacetate VI

(a) *Acetylation of desoxybostrycin* III. Bostrycin I (2 g) in 140 ml pyridine was hydrogenated with 5% pd-C (1·5 g). After air oxidation, Ac_2O (8·4 ml) was added and allowed to stand for 16 hr at room temp. After removal of the catalyst, the reaction mixture was concentrated to 30 ml *in vacuo*, and poured into ice–water (500 ml) to give 1·957 g of crude products. By chromatography on silica gel (100 g), 384 mg of VI was eluted with chloroform/acetone (4:1) as the main product. The diacetate crystallized as yellow needles from acetone/water; m.p. 206–209°. (Found: C, 59·46; H, 5·09. $C_{20}H_{20}O_9$ requires: C, 59·40; H, 4·99%); $[\alpha]_D^{22}$ −64·5 (*c*, 1·0, in pyridine); IR (KBr): 3460, 1764, 1740, 1712, 1638, 1608 cm^{-1}; IR ($CHCl_3$): 3670, 3580–3400, 1765, 1737, 1720 (sh), 1640, 1610 cm^{-1}; $\delta_{ppm}^{CDCl_3}$ 1·33 (3H, s, C-2-Me), 2·06 (1H, C-2-OH), 2·12 (3H, s, C-3-OAc), 2·41 (3H, s, C-5-OAc), 2·72-3·10 (4H, C-1-CH_2, C-4-CH_2), 3·89 [3·96]* (3H, C-7-OMe), 5·05 (1H, t, J = 6·0 c/s, C-3-H), 5·98 [6·68]* (1H, C-6-H), 12·49 [12·96]* (1H, C-8-OH).

(b) *Hydrogenolysis of triacetate* II. Triacetate II (1g) in 60 ml $CHCl_3$ absorbed about 2 moles H_2 upon hydrogenation over 5% pd-C (1 g). After removal of the catalyst, the solvent was evaporated *in vacuo* to dryness. Purification by silica gel chromatography, elution with chloroform/acetone (4:1), and crystallization from acetone/water gave 300 mg yellow needles which was identical with VI in all respects.

Anthraquinone derivatives VII, VIII *and* IX

Bostrycin (1 g) was refluxed in 50 ml formic acid (98–100%) for 8 hr to give 756 mg crude product which was poorly soluble in organic solvent, and treated with Ac_2O and pyridine. The crude acetate (757 mg) was chromatographed on silica gel (35 g).

Benzene/EtOAc (40:1) eluted 181 mg of the diacetate VII, which crystallized as yellow prisms; m.p. 238–243°. (Found: C, 65·25; H, 4·31. $C_{20}H_{16}O_7$ requires: C, 65·21; H, 4·38%); IR (KBr) 1770, 1675, 1597 cm^{-1} δ_{ppm}^{COCl} 2·47 (9H, s, C-2-Me, C-5, 6-OAc), 3·93 (3H, s, C-7-OMe), 6·91 (1H, s, C-6-H), 7·50 (1H, q, J = 7·6, 2·0 c/s, C-3-H), 7·94 (1H, d, J = 2 c/s, C-1-H), 8·05 (1H, d, J = 7·6 c/s, C-4-H).

Benzene/EtOAc (20:1) subsequently eluted 270 mg of VII which was crystallized from $CHCl_3$; m.p. 238–240°. (Found: C, 61·84; H, 4·28. $C_{22}H_{18}O_9$ requires: C, 61·97; H, 4·26%); IR (KBr) 1780, 1765, 1670, 1660, 1590, 1580 cm^{-1}; $\delta_{ppm}^{CDCl_3}$ 2·30 (3H, s, C-2-CH_3), 2·34 (3H, s, C-3-OAc), 2·47 (6H, s, C-5, -8-OAc), 3·93 (3H, s, C-7-OMe), 6·92 (1H, s, C-6-H), 7·81 (1H, s, C-4-H), 8·03 (1H, s, C-1-H).

Benzene/EtOAc (10:1) eluted 156 mg of IX which was crystallized from acetone–water; m.p. 220·5–221°. (Found: C, 59·61; F, 4·17. $C_{24}H_{20}O_{11}$ requires: C, 59·50; H, 4·16%); IR (KBr) 1780, 1675, 1495 cm^{-1}; $\delta_{ppm}^{CDCl_3}$ 2·31 (3H, s, C-2-Me), 2·34 (3H, s, C-3-OAc), 2·40 (6H, s, C-4, 5-OAc), 2·46 (3H, s, C-8-OAc), 3·90 (3H, s, C-7-OMe), 6·89 (1H, s, C-6-H), 7·98 (1H, s, C-1-H).

Debenzoate X *and monobenzoate* XI

After hydrogenation of bostrycin (1 g) in 70 ml pyridine with 10% Pd-C (1 g), 3·77 g *p*-bromobenzoylchloride in 50 ml pyridine was added and the mixture allowed to stand overnight in the absence of air. After removal of the catalyst, the filtrate was concentrated to about 10 ml. To this soln 100 ml water was added and extracted with chloroform. The organic layer was washed with $NaHCO_3$aq (50 ml × 2), 0·1N HCl (50 × 2) and dried over Na_2SO_4 and evaporated to dryness to give 1·503 g of crude benzoates which were chromatographed on silica gel (42 g) eluted with $CHCl_3$/acetone (19:1) to give 850 mg of amorphous X; IR (KBr) 3400, 1760, 1740, 1660, 1590 cm^{-1}; δ_{ppm}^{DMSO} 1·21 (3H, s, C-2-Me), 4·28–5·31 (3H, C-3, 3-OH, C-3-H), 8·20 (4H, d, J = 9 c/s, C-2'-H), 7·85 (4H, d, J = 9 c/s, C-1'-H) anl 523 mg of XI. The yellow XI crystallized as needles from EtOH; m.p. 193–196°. (Found: C, 56·72; H, 4·07; Br, 16·13. $C_{23}H_{19}O_7$ Br requires: C, 56·69; H, 3·93; Br, 16·40%); IR (KBr) 3400, 1755, 1655, 1600 cm^{-1}; λ_{max}^{EtOH} 267 (1·1 × 10^5), 278 (9·4 × 10^4), 376 mμ (ε 2·4 × 10^3); δ_{pp}^{DMSO} 1·18 (3H, s, C-2-Me), s, 4·2·7 (4H, C-1, 4-CH_2), 3·6 (1H, C-3-H), 3·95 (3H, s, C-7-OMe), 4·49 (1H, s, C-2-OH), 4·80 (1H, d, J = 4·5 c/s, C-3-OH), 7·30 (1H, d, J = 3 c/s, C-6-H), 7·45 (1H, d, J = 3 c/s, C-8-H), 7·85 (2H, d, J = 9 c/s, C-1'-H), 8·13 (2H, d, J = 9 c/s, C-2'-H).

Monobenzoate XI *from dibenzoate* X.

The dibenzoate (450 mg) in 20 ml pyridine was hydrogenated with 10% Pd-C (500 mg) and allowed to stand overnight in the absence of air. After removal of the catalyst, the filtrate was concentrated to 3 ml, and chromatographed on silica gel (15 g) and eluted with chloroform/acetone (10:1). From the first fraction 125 mg of the starting material (X) was recovered, then 94 mg of a yellow material which was identical with XI was eluted. The last polar fraction was concentrated to dryness to give 56 mg of a colorless product, which was identical with authentic *p*-bromobenzoic acid in all respects.

* The intensities of the peaks in parentheses are about ten per cent.

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