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ANTHRAQUINONES IN CELL SUSPENSION CULTURES OF *MORINDA CITRIFOLIA**

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Key Word Index—*Morinda citrifolia*; Rubiaceae; cell suspension culture; anthraquinones; anthraquinone primeverosides; droplet counter-current chromatography; ^{1}H NMR.

Abstract—From cell suspension cultures of *Morinda citrifolia* five known anthraquinones, rubiadin, lucidin, morindone, lucidin-3-β-primeveroside and morindone-6-β-primeveroside, and seven new anthraquinones were isolated. Six of the seven new quinones were characterized as 2-methyl-3,5,6-trihydroxyanthraquinone, 3-hydroxymorindone, 5,6-dihydroxylucidin, 2-methyl-3,5,6-trihydroxyanthraquinone-6-β-primeveroside, 3-hydroxymorindone-6-β-primeveroside and 5,6-dihydroxylucidin-3-β-primeveroside, respectively.

INTRODUCTION

Many plants of the Rubiaceae family have been shown to be rich sources of anthraquinones. For example, *Morinda citrifolia* L. contains thirteen anthraquinones, including glycosides [1]. Zenk and his coworkers [2] obtained from this plant, cell suspension cultures producing anthraquinones in high yields. Leistner [3] examined their constituents and isolated alizarin (**1**), rubiadin (**2**), lucidin (**3**), nordamnacanthal (**4**) and morindone (**5**), all of which had already been shown to be constituents of the original plant. We have re-examined the quinonoid constituents of these suspension cultures and isolated, besides the five known anthraquinones, seven new quinones, of which six have been structurally characterized. Although one and the same strain of *M. citrifolia* were used for this and the previous study [3], there is a considerable variance in the results.

RESULTS AND DISCUSSION

Extraction and isolation of quinones

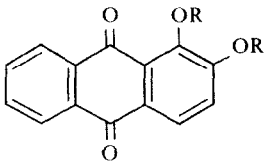
Cultured cells were extracted successively with benzene, tetrahydrofuran and methanol. As conventional adsorption chromatography techniques were inadequate to isolate the constituents from the extract, further fractionation was carried out by droplet counter-current chromatography (DCCC) [4], which is a form of liquid–liquid counter-current distribution chromatography. DCCC of the benzene extract with *n*-hexane–EtOH–H_2O–EtOAc (5:4:3:2) gave rubiadin (**2**), lucidin (**3**), morindone (**5**), 2-methyl-3,5,6-trihydroxyanthraquinone (**6**) and 3-hydroxymorindone (**7**). DCCC of the tetrahydrofuran extract using the solvent system just described yielded the same compounds as the benzene extract. Further DCCC with $CHCl_3$–MeOH–H_2O (5:5:3) gave 5,6-dihydroxylucidin (**8**), lucidine-3-β-primeveroside (**9**), morindone-6-β-primeveroside (**10**) and 3-hydroxymorindone-6-β-primeveroside (**11**). DCCC of the methanol extract with EtOAc–*n*-PrOH–H_2O (7:3:9) gave lucidin-3-β-primeveroside (**9**), 2-methyl-3,5,6-trihydroxyanthraquinone-6-β-primeveroside (**12**), 5,6-dihydroxylucidin-3-β-primeveroside (**13**), a mixture of **10** and **11**, and an undefined dimeric quinone. Finally, **10** and **11** were separated by DCCC with $CHCl_3$–MeOH–H_2O (5:5:3). Substances **2**, **3**, **5**, **9** and **10**, were identified by comparisons with authentic samples (mp, UV, IR, NMR and, in the case of **10**, $[\alpha]_D$). The other quinones were new compounds. In the present experiments, however, alizarin (**1**) and nordamnacanthal (**4**), two anthraquinones isolated by Leistner [3] from the same cell strain, were not detected.

Structures of the new anthraquinones

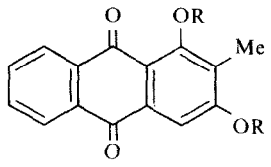
2-Methyl-3,5,6-trihydroxyanthraquinone (**6**), $C_{15}H_{10}O_5$, orange-red needles, mp > 300°, exhibited UV absorption maxima (MeOH) at 222, 279, 338 and 414 nm (log ε 4.30, 4.65, 3.71 and 3.74) and IR bands (KBr) at 3440, 1630 and 1570 cm^{-1}. Its ^{1}H NMR spectrum (DMSO-d_6, 100 MHz) contained a singlet (δ 2.26) due to a benzylic methyl group, a pair of doublets (δ 7.12 and 7.53, J = 8.0 Hz) due to two *ortho*-positioned aromatic protons, and a pair of singlets (δ 7.48 and 7.82) due to *para*-positioned aromatic protons. It also showed a broad singlet around δ 10.80 assignable to a phenolic hydroxy group hydrogen-bonded to a carbonyl group. This signal disappeared on treatment with D_2O.

Conventional acetylation of **6** gave the triacetate (**27**). The positions of the acetoxy and methyl groups of this compound (**6**) were assigned by comparisons of the chemical shifts of these substituents with those of several other compounds of defined structures.

* Part 12 in the series "Quinones and Related Compounds in Higher Plants (H. Inouye)". For Part 11 see Inoue, K., Ueda, S., Shiobara, Y., Kimura, I. and Inouye, H. (1981) *J. Chem. Soc. Perkin Trans.* 1, (in press).



1 R = H
19 R = Ac



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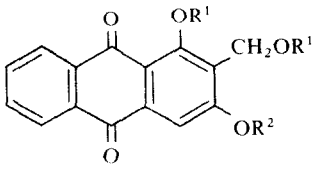
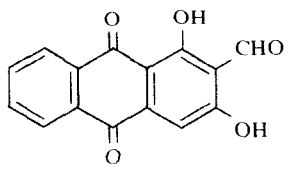
22 R = Ac



3 $R^1 = R^2 = H$
9 $R^1 = H$, R^2 = prim H_6
23 $R^1 = R^2$ = Ac
24 R^1 = Ac, R^2 = prim Ac_6



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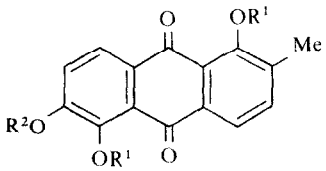
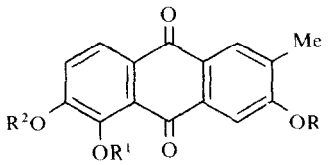
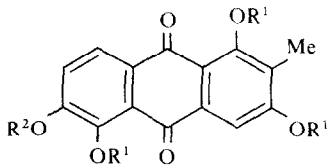


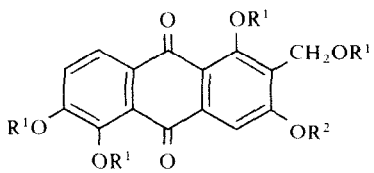
5 $R^1 = R^2 = H$
10 $R^1 = H$, R^2 = prim H_6
25 $R^1 = R^2$ = Ac
26 R^1 = Ac, R^2 = prim Ac_6



6 $R^1 = R^2 = H$
12 $R^1 = H$, R^2 = prim H_6
27 $R^1 = R^2$ = Ac
28 R^1 = Ac, R^2 = prim Ac_6

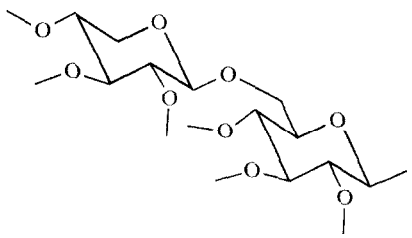


7 $R^1 = R^2 = H$
11 $R^1 = H$, R^2 = prim H_6
29 $R^1 = R^2$ = Ac
30 R^1 = Ac, R^2 = prim Ac_6

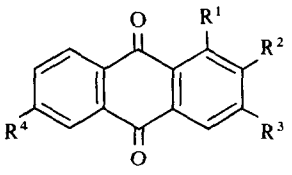


8 $R^1 = R^2 = H$
13 $R^1 = H$, R^2 = prim H_6
31 $R^1 = R^2$ = Ac
32 R^1 = Ac, R^2 = prim Ac_6

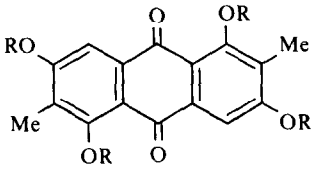
prim =



The chemical shifts of the ^1H NMR signals of 2-methylanthraquinone (**14**), 1-acetoxy-2-methylanthraquinone (**15**), 3-acetoxy-2-methylanthraquinone (**16**), 1-acetoxyanthraquinone (**17**), 2-acetoxyanthraquinone (**18**), diacetylalizarin (**19**), 2-acetoxymethyl-1,3,6-triacetoxyanthraquinone (**20**), 1,3,5,7-tetraacetoxy-2,6-dimethylanthraquinone (**21**) (see later) as well as those of the acetates **22**–**26** of rubiadin (**2**), lucidin (**3**), lucidin-3-β-primeveroside (**9**), morindone (**5**) and morindone-6-β-primeveroside (**10**) are shown in Table 1 together with those of the acetates **27**–**32** of the new quinones and quinone glycosides.



14 $R^1 = R^3 = R^4 = H$, $R^2 = Me$
15 $R^1 = OAc$, $R^2 = Me$, $R^3 = R^4 = H$
16 $R^1 = R^4 = H$, $R^2 = Me$, $R^3 = OAc$
17 $R^1 = OAc$, $R^2 = R^3 = R^4 = H$
18 $R^1 = R^3 = R^4 = H$, $R^2 = OAc$
19 $R^1 = R^2 = OAc$, $R^3 = R^4 = H$
20 $R^1 = R^3 = R^4 = OAc$, $R^2 = CH_2OAc$



21 R = OAc
33 R = H

Detailed examination of the chemical shifts of acetoxy and methyl groups of the known compounds **14**–**26** revealed the following regularities: In anthraquinones that have a methyl or an acetoxymethyl group at C-2, the signal of the C-1 acetoxy group appeared at about δ 2.50, while that of the C-3 acetoxy group appeared at about δ 2.38. The signal of the C-2 methyl group appeared at δ 2.14 when both C-1 and C-3 were substituted by acetoxy groups, whereas it was at about δ 2.33 when only one of these carbon atoms was substituted by an acetoxy group. In anthraquinones having *ortho*-substituted diacetoxy groups at C-1 and C-2 (or C-5 and C-6), the signal of the acetoxy group on C-1 (or C-5) appeared at δ 2.47, while that of the acetoxy group on C-2 (or C-6) appeared at δ 2.34. Accordingly, the broad singlet at δ 2.32 and the singlet at δ 2.37 in the spectrum of the acetate (**27**) were assigned to the C-2 methyl and the C-3 acetoxy groups, and the signals at δ 2.34 and 2.47 to the C-6 and C-5, or C-7 and C-8 acetoxy groups, respectively. It was thus presumed that the two *para* protons occupied the 1 and 4 positions and the two *ortho* protons the 7 and 8 or 5 and 6 positions. Taking into account the structure of morindone (**5**) together with the above-mentioned presumption, it was further inferred that the acetate (**27**) was 2-methyl-3,5,6-triacetoxyanthraquinone and that the original quinone was 2-methyl-3,5,6-trihydroxyanthraquinone (**6**). This inferrence was verified by the demonstration that the 2-methyl-3,5,6-trihydroxyanthraquinone [5], obtained by the condensation of opianic acid with *o*-cresol, and its acetate were identical with **6** and **27**, respectively.

3-Hydroxymorindone (**7**), $C_{15}H_{10}O_6$, red needles, mp >300°, exhibited UV absorption maxima (MeOH) at 227, 276, 316 and 450 nm (log ε 4.27, 4.43, 3.95 and 3.90) and IR bands at 3420 and 1595 cm^{-1}. The carbonyl band showed a considerable bathochromic shift in comparison with those of ordinary anthraquinones which suggested that both quinone carbonyls were hydrogen-bonded. In the ^1H NMR spectrum, it showed a singlet (δ 2.03) due to a benzylic methyl group, a pair of doublets (δ 7.13 and 7.58, $J = 8.0$ Hz) assignable to two adjacent aromatic protons, a singlet (δ 7.20) of another aromatic proton and a singlet (δ 13.33) of a hydrogen-bonded phenolic hydroxy proton. These findings together with the results of the examination of the ^1H NMR signals of its acetate (**29**), in the same way as described above, suggested that **29** was 2-methyl-1,3,5,6-tetraacetoxyanthraquinone, and hence **7** was 3-hydroxymorindone. This was proved by the synthesis of **7** by the condensation of protocatechuic acid with 3,5-dihydroxy-*p*-toluic acid in the presence of concentrated sulfuric acid. 2,6-Dimethyl-1,3,5,7-tetrahydroxyanthraquinone (**33**) was obtained as the main product of this condensation, the structure of which was verified from the synthetic route and the ^1H NMR spectral data of its acetate (**21**).

5,6-Dihydroxylucidin (**8**), $C_{15}H_{10}O_7$, red needles, mp >300°, showed UV absorption maxima at 233, 268, 288, 315 (sh.) and 446 nm (log ε 4.45, 4.45, 4.12, 3.95 and 3.93) and IR bands at 3350 and 1600 cm^{-1}. Its ^1H NMR spectrum, contained a signal for benzylic methylene protons at δ 4.42 in place of the benzylic methyl signal shown by **7** at δ 2.03. Furthermore, its acetate (**31**) showed ^1H NMR signals which were almost superimposable on those of **29**, except for the signals due to the replacement of the methyl group by an acetoxymethyl group. **8** was thus assumed to be 5,6-dihydroxylucidin. This assumption was verified by the identification of **31** and **8** with the acetoxy compound derived from 3-hydroxymorindone tetraacetate (**29**) through treatment with NBS followed by the work-up with acetic anhydride–sodium acetate, and the hydrolysate of **29**, respectively.

Structures of the new anthraquinone glycosides

2-Methyl-3,5,6-trihydroxyanthraquinone-6-β-primeveroside (**12**), $C_{26}H_{28}O_{14} \cdot H_2O$, yellow needles, mp 269–271°, $[\alpha]_D$ −36.4° (DMF). The UV spectrum of this compound was very similar to that of **6**. Exhaustive hydrolysis of this compound with dilute hydrochloric acid gave glucose and xylose in addition to **6**. In contrast, hydrolysis of **12** in H_2O under UV irradiation gave primeverose as a sole sugar. [Under the same conditions, crystalline primeverose was obtained from lucidin-3-β-

Table 1. ^{1}H NMR data of anthraquinone acetates **14**–**32** (60 MHz, $CDCl_3$, TMS as internal standard)

Compound	Position*							
	1	2	3	4	5	6	7	8
14		2.50 (Me)						
15	2.51 (OAc)	2.33 (Me)						
16		2.34 (Me)	2.38 (OAc)					
17					2.47 (OAc)			
18						2.35 (OAc)		
19	2.47 (OAc)	2.34 (OAc)						
20	2.49 (OAc)	1.99 ($CH_2O\underline{Ac}$)	2.38 (OAc)	7.96 (*s*)	7.89 (*d*, J = 2 Hz)	2.33 (OAc)	7.44 (*dd*, J = 2,8 Hz)	8.21 (*d*, J = 8 Hz)
21	2.50 (OAc)	2.14 (Me)	2.37 (OAc)	7.90 (*s*)	2.50 (OAc)	2.14 (CH_3)	2.37 (OAc)	7.90 (*s*
22	2.52 (OAc)	2.15 (Me)	2.38 (OAc)	7.97 (*s*)	8.13–8.32 (*m*)	7.65–7.87 (*m*)	7.65–7.87 (*m*)	8.13–8.32 (*m*)
23	2.52 (OAc)	2.00 ($CH_2O\underline{Ac}$)	2.39 (OAc)	8.03 (*s*)	8.13–8.31 (*m*)	7.67–7.87 (*m*)	7.67–7.87 (*m*)	8.13–8.31 (*m*)
24	2.50 (OAc)	1.90 ($CH_2O\underline{Ac}$)	—	7.89 (*s*)	8.15–8.57 (*m*)	7.68–7.83 (*m*)	7.68–7.83 (*m*)	8.15–8.57 (*m*)
25	2.50 (OAc)	2.33 (Me)	7.60 (*d*, J = 8 Hz)	8.03 (*d*, J = 8 Hz)	2.47 (OAc)	2.34 (OAc)	7.53 (*d*, J = 8 Hz)	8.17 (*d*, J = 8 Hz)
26	2.50 (OAc)	2.32 (Me)	7.62 (*d*, J = 8 Hz)	8.03 (*d*, J = 8 Hz)	2.43 (OAc)	—	7.38 (*d*, J = 8 Hz)	8.19 (*d*, J = 8 Hz)
27	8.10 (*s*)	2.32 (Me)	2.37 (OAc)	7.82 (*s*)	2.47 (OAc)	2.34 (OAc)	7.57 (*d*, J = 8 Hz)	8.25 (*d*, J = 8 Hz)
28	8.13 (*s*)	2.32 (Me)	2.36 (OAc)	7.83 (*s*)	2.43 (OAc)	—	7.43 (*d*, J = 8 Hz)	8.33 (*d*, J = 8 Hz)
29	2.50 (OAc)	2.14 (Me)	2.37 (OAc)	7.88 (*s*)	2.45 (OAc)	2.34 (OAc)	7.58 (*d*, J = 8 Hz)	8.20 (*d*, J = 8 Hz)
30	2.50 (OAc)	2.14 (Me)	2.37 (OAc)	7.87 (*s*)	2.42 (OAc)	—	7.40 (*d*, J = 9 Hz)	8.18 (*d*, J = 9 Hz)
31	2.50 (OAc)	2.00 ($CH_2O\underline{Ac}$)	2.37 (OAc)	7.88 (*s*)	2.45 (OAc)	2.36 (OAc)	7.57 (*d*, J = 8 Hz)	8.20 (*d*, J = 8 Hz)
32	2.49 (OAc)	1.90 ($CH_2O\underline{Ac}$)	—	7.73 (*s*)	2.46 (OAc)	2.33 (OAc)	7.48 (*d*, J = 8 Hz)	8.17 (*d*, J = 8 Hz)

* Chemical shifts designated together with bracketed Me, OAc or $CH_2O\underline{Ac}$ are those of the Me, OAc or $CH_2O\underline{Ac}$ substituents at these positions. Unspecified figures are the chemical shifts of the protons at the denoted positions.

primeveroside (**9**) (Prof. K. Takaishi, Faculty of Pharmaceutical Sciences, Kinki University, private communication).] The ^{1}H NMR spectrum of the sugar moiety of its acetate (**28**) was the same as that of **26** which suggested a β-glucosidic linkage between the sugar and phenolic hydroxy group. Although the chemical shifts of the signals of the C-5 acetoxy groups of **27** and **28** were about the same, the signal at δ 2.34 of **27** was assigned to either the C-3 or C-6 acetoxy group, as the signals of both acetoxy groups in this series of substances are close to each other (Table 1). Thus, it was invalid to infer the binding site of the sugar only from the absence of the signals in question. Further detailed comparison of the chemical shifts of lucidin acetate (**23**) with those of lucidin-3-β-primeveroside acetate (**24**), as well as morindone acetate (**25**) with those of morindone-6-β-primeveroside acetate (**26**) (Table 1) revealed, however, that the signal of the aromatic proton adjacent to the glucosyloxy-bearing carbon (C-4 proton of **24** and C-7 proton of 26) appeared in the upper field at *ca* 0.15 ppm higher than that due to the proton adjacent to the acetoxy-bearing carbon (C-4 proton of **23** and C-7 proton of **25**). These results showed that the binding site of the sugar could be inferred from the chemical shift of the aromatic proton concerned. The upfield shift of the aromatic proton on C-7 of **28** by 0.14 ppm relative to the corresponding proton of **27** meant, therefore, that the sugar was bound to the C-6 hydroxy group in **28**, and hence in **12**.

3-Hydroxymorindone-6-β-primeveroside (**11**), $C_{26}H_{28}O_{15}\cdot H_2O$, orange-yellow needles, mp 282–284°, $[\alpha]_D$ −69.6° (DMF). GLC of the acid hydrolysate of this substance revealed the presence of glucose and xylose besides **7**, whereas hydrolysis under irradiation with UV light afforded primeverose as a sole sugar. The ^{1}H NMR spectrum of the sugar moiety of its acetate (**30**) was identical with those of **26** and **28** and suggested a β-glucosidic linkage between the sugar and phenolic hydroxy group. This binding site for the sugar was also supported by the ^{1}H NMR spectra of the acetates **29** and **30** derived from **7** and **11**, respectively.

5,6-Dihydroxylucidin-3-β-primeveroside (**13**), $C_{26}H_{28}O_{16}\cdot H_2O$, red needles, mp 180–182°, $[\alpha]_D$ −50.0° (DMF). The structure of this compound was assigned in the same way as described above by the formation of **8**, glucose and xylose on acid hydrolysis, and the production of primeverose under UV irradiation in H_2O. The location of the sugar on the C-3 hydroxy group was assigned by comparing the ^{1}H NMR spectra of **32** and **31**. Furthermore, the β-glucosidic linkage between the sugar and phenolic hydroxy group was suggested from the comparison of the ^{1}H NMR spectra of **32** and **24**, whose signal patterns of the sugar moiety were superimposable.

The unidentified substance, $C_{52}H_{54}O_{29}\cdot 4H_2O$, yellow needles, mp 262–264°, $[\alpha]_D$ −105.9° (DMF), was assumed to be a dimeric compound consisting of two anthraquinone primeverosides from its elemental composition, spectral properties (including FDMS) and the nature of its hydrolytic products. Its structure was not elucidated because of the limited amount of sample available for analysis.

All of the variously hydroxylated anthraquinones described above are considered to be formed via 2-succinylbenzoic acid and 2-prenylnaphthohydroquinone-3-carboxylic acid [6]. Elucidation of the cyclization mechanism from the latter and the sequence of hydroxylation in aromatic rings of anthraquinones are intriguing problems to be solved.

EXPERIMENTAL

General procedures. Mps were uncorr. Unless otherwise stated, ^{1}H NMR spectra were measured at 60 MHz in $CDCl_3$ and the chemical shifts are given in δ values (ppm) with TMS as the int. standard. Si gel GF_{254} was employed for TLC and spots were visualized, when necessary, by exposure to I_2 or under UV radiation (254 nm). Si gel PF_{254} was used for prep. TLC and bands were visualized, when necessary, under UV radiation. MS was obtained by direct inlet; FD mode, emitter current 18–20 mA, accelerating voltage 2 kV. GLC were carried out using glass columns (2 m × 6 mm) packed with 3% OV-1 on 100–200 mesh Gas Chrom Q or with 1.5% OV-17 on 80–100 mesh Shimalite W, using N_2 as carrier gas (45 ml/min). Droplet counter-current chromatography (DCCC) was carried out under the following conditions: DCCC-A, ascending method with a solvent system, *n*-hexane–EtOH–H_2O–EtOAc (5:4:1:2) using Pyrex glass tubes (120 cm × 2.4 mm i.d.) connected to each other by Teflon tubings (140 cm × 0.65 mm i.d.); DCCC-B, descending method with $CHCl_3$–MeOH–H_2O (5:5:3) using Pyrex glass tubes (120 cm × 2.4 mm i.d.) connected by Teflon tubings (140 cm × 0.86 mm i.d.); DCCC-C, ascending method with EtOAc–*n*-PrOH–H_2O (7:3:9) using Pyrex glass tubes (120 cm × 4.2 mm i.d.) connected by Teflon tubings (140 cm × 1.35 mm i.d.).

Cultivation of callus cells and extraction of their components. Cell suspension cultures of *Morinda citrifolia* L. were grown in B5 medium developed by Gamborg *et al.* [7] containing 6% sucrose and as hormone 10^{-5} M 2,3-dimethoxyphenoxyacetic acid and 0.2% N-Z-amine type A (Sheffield Chemical, U.K.) for 2 weeks. Cells were collected by filtration, freeze-dried (wt 55 g) and extracted successively with C_6H_6 (300 ml × 3), THF (500 ml × 3) and MeOH (500 ml × 3) at room temp. under vigorous agitation. The C_6H_6, THF and MeOH extracts were concd *in vacuo* to give residues of 0.67, 3.95 and 31.91 g, respectively.

Isolation of free anthraquinones. The C_6H_6 extract (0.67 g) was fractionated by DCCC-A (52 glass tubes), and 7 ml fractions (fr.) were collected. Fr. 5–30 were combined and concd *in vacuo* to give a residue (151.4 mg), which was purified by prep. TLC (C_6H_6) followed by recrystallization from MeOH to give colourless needles (25 mg) of β-sitosterol (mp 138–139°), which were identified by comparison with an authentic sample (mmp, IR, NMR). The combined fr. 51–55 were concd to give a residue (37.6 mg), which was subjected to prep. TLC (C_6H_6–EtOAc, 4:1). The main orange-yellow band (R_f 0.70) gave, on treatment in the usual way followed by recrystallization from C_6H_6, rubiadin (**2**) (7 mg) as yellow plates. Fr. 56–90 on concn gave a residue (74 mg), which was recrystallized from MeOH to give 3-hydroxymorindone (**7**) (12 mg) as red needles. The combined fr. 91–145 on concn gave a residue (82 mg), which was recrystallized from MeOH to give 2-methyl-3,5,6-trihydroxyanthraquinone (**6**) (5.8 mg) as orange-red needles. The mother liquors from the recrystallizations of **6** and **7** were combined and concd *in vacuo* to give a residue (120 mg), which was subjected to prep. TLC (Si gel treated with 0.25% orthophosphoric acid; C_6H_6–EtOAc, 4:1). The main orange-yellow band (R_f 0.51) afforded on treatment in the usual way, followed by recrystallization from MeOH, morindone (**5**) (4.5 mg) as orange needles.

The THF extract (3.95 g) on trituration in MeOH gave insoluble yellow crystals which were recrystallized from C_6H_6 to give lucidin (**3**) (30 mg) as yellow needles. The MeOH soln was concd *in vacuo* to give a residue (3.85 g), which was subjected to DCCC-A in the same way as above. 3-Hydroxymorindone (**7**) (5 mg) and 2-methyl-3,5,6-trihydroxyanthraquinone (**6**) (3 mg)

were obtained from combined fr. 40–75 (53.2 mg) and fr. 76–145 (143.1 mg), respectively. Morindone (**5**) (3.5 mg) was also obtained from the mother liquor of **6** by prep. TLC worked up in the same way as mentioned above. The combined fr. 146–250 on concn gave a residue (63.9 mg), which was recrystallized from DMSO–MeOH to give 5,6-dihydroxylucidin (**8**) (6 mg) as red needles.

2, orange plates, mp > 300°; UV λ_{max}^{MeOH} nm (log ε): 241 (4.38), 245 (4.41), 278.5 (4.49), 411 (3.94); IR ν_{max}^{KBr} cm^{-1}: 3370, 1625, 1608, 1590; ^{1}H NMR (DMSO-d_6): δ 1.97 (3H, *s*, benzyl Me), 7.10 (1H, *s*, H-4), 7.70–8.17 (4H, *m*, H-5, 6, 7, 8), 12.94 (1H, *s*, —OH, disappeared on treatment with D_2O). [Found: C, 70.98; H, 3.98. Calc. for $C_{15}H_{10}O_4$: C, 70.85; H, 3.97%]. This substance was identical with an authentic sample (IR, NMR).

3, yellow needles, mp > 300°; UV λ_{max}^{MeOH} nm (log ε): 242 (4.34), 246 (4.35), 280 (4.35), 332 (3.40), 410 (3.75); IR ν_{max}^{KBr} cm^{-1}: 3380, 1655, 1610, 1585; ^{1}H NMR (DMSO-d_6): δ 4.50 (2H, *s* benzyl —CH_2—), 7.16 (1H, *s* H-4), 7.72–8.23 (4H, *m*, H-5, 6, 7, 8), 13.07 (1H, *s*, —OH, disappeared on treatment with D_2O). [Found: C, 66.45; H, 3.81. Calc. for $C_{15}H_{10}O_5$: C, 66.67; H, 3.73%]. This substance was identified with an authentic sample (IR, NMR).

5, orange needles, mp 283–284°; UV λ_{max}^{MeOH} nm (log ε): 232 (4.49), 260 (4.50), 293 (4.18), 335 (3.42), 432 (3.78); IR ν_{max}^{KBr} cm^{-1}: 3440, 1630, 1605; ^{1}H NMR (DMSO-d_6, 100 MHz): δ 2.27 (3H, *s* benzyl Me), 7.15 (1H, *d* J = 8.0 Hz, H-7), 7.53 (1H, *d* J = 8.0 Hz, H-3), 7.75 (1H, *d* J = 8.0 Hz, H-4), 8.05 (1H, *d*, J = 8.0 Hz, H-8). [Found: C, 66.72; H, 3.68. Calc. for $C_{15}H_{10}O_5$: C, 66.67; H, 3.73%]. This substance was identified with an authentic sample (mmp, IR).

6, orange needles, mp > 300°. [Found: C, 66.59; H, 3.88. $C_{15}H_{10}O_5$ requires: C, 66.67; H, 3.73%].

7, red needles, mp > 300°. [Found: C, 62.87; H, 3.54. $C_{15}H_{10}O_6$ requires: C, 62.95; H, 3.52%].

8, red needles, mp > 300°; ^{1}H NMR (DMSO-d_6): δ 4.42 (2H, *s* benzyl —CH_2—), 7.14 (1H, *d*, J = 8.0 Hz, H-7), 7.25 (1H, *s*, H-4), 7.60 (1H, *d*, J = 8.0 Hz, H-8), 10.70, 12.50 (each 1H, *s*(*br*), —OH), 13.48 (1H, *s*, —OH). [Found: C, 59.58; H, 3.46. $C_{15}H_{10}O_7$ requires: C, 59.62; H, 3.33%].

Isolation of anthraquinone glycosides. The stationary phases left in glass tubes after DCCC-A of the THF extract were combined and concd *in vacuo* to give a residue (3.4 g), which was further subjected to DCCC-B (78 glass tubes) collecting 6 ml fractions. Fr. 101–155 were combined and concd *in vacuo* to *ca* 120 ml. The resulting orange-yellow ppt. was collected, washed with H_2O and recrystallized repeatedly from DMSO–MeOH to give morindone-6-β-primeveroside (**10**) (34 mg) as orange-yellow needles. Fr. 301–500 were combined and concd *in vacuo* to *ca* 1/3 of the original vol. The resulting orange ppt. was collected and recrystallized repeatedly from DMSO–MeOH to give lucidin-3-β-primeveroside (**9**) (30 mg) as yellow needles.

An aliquot (5 g) of the MeOH extract was fractionated by DCCC-C (100 glass tubes) and 7 ml fractions were collected. Fr. 1–20 were combined and concd *in vacuo* to give a residue (91.4 mg), which was recrystallized from C_6H_6 to give lucidin (**3**) (3.2 mg) as yellow needles. Combined fr. 44–100 gave on concn *in vacuo* a residue (80 mg). Combined fr. 101–150 gave a residue (30.3 mg) in the same way, which was triturated in MeOH and the insoluble material was recrystallized repeatedly from DMSO–MeOH to give 2-methyl-3,5,6-trihydroxyanthraquinone-6-β-primeveroside (**12**) (8.8 mg) as orange-yellow needles. Fr. 185–270 gave on concn *in vacuo* a yellow crystalline residue, which was recrystallized from DMSO–MeOH to give lucidin-3-β-primeveroside (**9**) (11.2 mg) as yellow needles. A residue obtained from fr. 341–400 (9.1 mg) gave, on recrystallization from MeOH–H_2O, 5,6-dihydroxylucidin-3-β-primeveroside (**13**) (12.5 mg) as red needles. The stationary phases left in glass tubes at the end of the DCCC separation were combined and concd *in vacuo*. The residue was triturated with H_2O and the resulting yellow ppt. was recrystallized from DMSO–MeOH to give the dimeric substance (4 mg) as yellow needles. Combined fr. 44–100 were further subjected to DCCC-B (27 glass tubes) and 7 ml fractions were collected. Fr. 16–70 gave on concn *in vacuo* an orange-yellow residue, which was recrystallized from DMSO–MeOH to give morindone-6-β-primeveroside (**10**) (5 mg) as orange-yellow needles. From fr. 71–140 3-hydroxymorindone-6-β-primeveroside (**11**) (3.2 mg) was obtained as orange needles. The remaining aliquot of fr. 44–100 was also treated in the same way as mentioned above resulting in the isolation of six glycosides.

9, yellow needles, mp 210–212°; $[\alpha]_D^{20}$ −103.5° (*c* 1.7, DMF); UV λ_{max}^{MeOH} nm (log ε): 242 (4.34), 246 (4.36), 266 (4.39), 406 (3.79); IR ν_{max}^{KBr} cm^{-1}: 3400, 1670, 1630, 1595; ^{1}H NMR (DMSO-d_6): δ 4.65 (2H, *s*, benzyl —CH_2—), 3.22–5.22 (13H, *m*, sugar protons), 7.47 (1H, *s* H-4), 7.79–8.23 (4H, *m*, H-5,6,7,8), 13.01 (1H, *s*, —OH). [Found: C, 53.50; H, 5.08. Calc. for $C_{26}H_{28}O_{14}\cdot H_2O$: C, 53.62; H, 5.19%]. The properties of this substance were in agreement with those reported in the literature [8, 9] (mp, IR, $[\alpha]_D$).

10, orange-yellow needles, mp 264–266°; $[\alpha]_D^{20}$ −84.9° (*c* 0.11, DMF). [Found: C, 53.74; H, 5.21. Calc. for $C_{26}H_{28}O_{14}\cdot H_2O$: C, 53.26; H, 5.19%]. This substance was identified by direct comparison with an authentic sample [10] (mp, IR, $[\alpha]_D$).

11, orange needles, mp 282–284°; $[\alpha]_D^{20}$ −69.6° (*c* 0.23, DMF). [Found: C, 52.47; H, 5.31; $C_{26}H_{28}O_{15}\cdot H_2O$ requires: C, 52.18; H, 5.05%].

12, orange needles, mp 269–271°; $[\alpha]_D^{20}$ −36.4° (*c* 0.11, DMF); UV λ_{max}^{MeOH} nm (log ε): 225 (4.45), 264.5 (4.45), 280 (4.56), 420 (3.97); IR ν_{max}^{KBr} cm^{-1}: 3400, 1640, 1620, 1575. [Found: C, 53.54; H, 5.38. $C_{26}H_{28}O_{14}\cdot H_2O$ requires: C, 53.62; H, 5.19%].

13, red needles, mp 180–182°; $[\alpha]_D^{20}$ −50.0° (*c* 0.1, DMF); UV λ_{max}^{MeOH} nm (log ε): 232 (4.46), 266.5 (4.45), 288 (sh) (4.18), 311 (sh) (3.94), 446 (3.99); IR ν_{max}^{KBr} cm^{-1}: 3350, 1660, 1620, 1590; ^{1}H NMR (DMSO-d_6, 100 MHz): δ 3.94 (2H, *s*, benzyl —CH_2—), 2.95–5.09 (13H, *m*, sugar protons), 7.37 (1H, *d*, J = 8.0 Hz, H-7), 7.48 (1H, *s*, H-4), 7.72 (1H, *d*, J = 8.0 Hz, H-8), 12.63, 13.28 (each 1H, *s*, —OH). [Found: C, 48.76; H, 5.10. $C_{26}H_{28}O_{16}\cdot H_2O$ requires: C, 48.56; H, 4.89%].

Dimeric substance, yellow needles, mp 262–264°; $[\alpha]_D^{20}$ −105.9° (*c* 0.17, DMF); UV λ_{max}^{MeOH} nm (log ε): 223 (4.55), 280 (4.72), 416 (4.11); IR ν_{max}^{KBr} cm^{-1}: 3400, 1615, 1600, 1580; ^{1}H NMR (DMSO-d_6): δ 2.07, 2.29 (each 3H, *s*, 2 × benzyl Me), 2.80–5.50 (26H, *m*, sugar protons), 7.28–7.93 (7H, *m*, arom. protons), 11.10 (1H, *s*(*br*), —OH), 12.75 and 13.32 (each 1H, *s*, —OH); FDMS Found: M^+ +23, 1165. $C_{52}H_{54}O_{29}\cdot Na$ requires: 1165. [Found: C, 51.71; H, 4.94. $C_{52}H_{54}O_{29}\cdot 4H_2O$ requires: C, 51.41; H, 5.14%.]

Acetylation of isolated free anthraquinones. **2** (5 mg), **3** (10 mg), **5** (4 mg), **6** (10 mg), **7** (5 mg) and **8** (5 mg) were acetylated with Ac_2O and pyridine in the usual way and the products were recrystallized from MeOH to furnish **22** (5 mg), **23** (11 mg), **25** (4.2 mg), **27** (12 mg), **29** (5 mg) and **31** (7 mg), respectively.

Rubiadin diacetate (**22**), pale yellow needles, mp 225°. UV λ_{max}^{MeOH} nm (log ε): 210 (4.44), 257.5 (4.68), 274 (sh) (4.19), 330 (3.75); IR ν_{max}^{KBr} cm^{-1}: 1756, 1665, 1585. [Found: C, 67.22; H, 4.29. Calc. for $C_{19}H_{14}O_6$: C, 67.44; H, 4.17%].

Lucidin triacetate (**23**), pale yellow needles, mp 169–170°; UV λ_{max}^{MeOH} nm (log ε): 257.5 (4.72), 273 (4.22), 330 (3.77); IR ν_{max}^{KBr} cm^{-1}: 1765, 1740, 1670, 1585. [Found: C, 63.94; H, 4.35. Calc. for $C_{21}H_{16}O_8$: C, 63.64; H, 4.07%].

Morindone triacetate (**25**), pale yellow needles, mp 255–257°. UV λ_{max}^{MeOH} nm (log ε): 211 (4.45), 256.5 (4.53), 275(sh) (4.14), 338 (3.77); IR ν_{max}^{KBr} cm^{-1}: 1765, 1670, 1585. [Found: C, 63.51; H, 3.89. Calc. for $C_{21}H_{16}O_8$: C, 63.64; H, 4.07%].

2-Methyl-3,5,6-trihydroxyanthraquinone triacetate (**27**), pale yellow needles, mp 229–231°. UV λ_{max}^{MeOH} nm (log ε): 207.5 (4.42), 259 (4.63), 276(sh) (4.28), 334 (3.84); IR ν_{max}^{KBr} cm^{-1}: 1765, 1665, 1585. [Found: C, 63.72; H, 4.05. $C_{21}H_{16}O_8$ requires: C, 63.64; H, 4.07%].

3-Hydroxymorindone tetraacetate (**29**), pale yellow needles, mp 255–256°. UV λ_{max}^{MeOH} nm (log ε): 210 (4.15), 259.5 (4.65), 278 (4.16), 335 (3.84); IR ν_{max}^{KBr} cm^{-1}: 1750, 1660, 1580. [Found: C, 58.74; H, 3.85. $C_{25}H_{20}O_{12}$ requires: C, 58.61; H, 3.93%].

5,6-Dihydroxylucidin pentaacetate (**31**), pale yellow needles, mp 222–224°. UV λ_{max}^{MeOH} nm (log ε): 259 (4.55), 277(sh) (4.09). 334 (3.75); IR ν_{max}^{KBr} cm^{-1}: 1770, 1670, 1585. [Found: C, 58.44; H, 3.86. $C_{25}H_{20}O_{12}$ requires: C, 58.61; H, 3.93%].

Acetylation of anthraquinone glycosides. **9** (20 mg), **10** (15 mg), **11** (15 mg), **12** (10 mg) and **13** (6 mg) were acetylated with Ac_2O and pyridine in the usual manner and the products were recrystallized from MeOH to give **24** (20 mg), **26** (17 mg), **30** (11 mg), **28** (17 mg) and **32** (7 mg), respectively.

Lucidin-3-β-primeveroside octaacetate (**24**), yellow needles, mp 191–192°; UV λ_{max}^{MeOH} nm (log ε): 262.5 (4.62), 331 (3.72); IR ν_{max}^{KBr} cm^{-1}: 1740, 1670, 1585. [Found: C, 55.04; H, 4.79. Calc. for $C_{42}H_{44}O_{22} \cdot H_2O$: C, 54.91; H, 5.05%].

Morindone-6-β-primeveroside octaacetate (**26**), pale yellow needles, mp 265–267°; UV λ_{max}^{MeOH} nm (log ε): 261 (4.58), 281.5 (4.27), 342 (3.88); IR ν_{max}^{KBr} cm^{-1}: 1750, 1670, 1590. [Found: C, 55.99; H, 5.10. Calc. for $C_{42}H_{44}O_{22}$: C, 56.01; H, 4.92%].

2-Methyl-3,5,6-trihydroxyanthraquinone-6-β-primeveroside octaacetate (**28**), pale yellow needles, mp 229–231°. UV λ_{max}^{MeOH} nm (log ε): 264.5 (4.76), 280.5 (4.35), 330 (3.78); IR ν_{max}^{KBr} cm^{-1}: 1750, 1740, 1665, 1595. [Found: C, 56.10; H, 5.12. $C_{42}H_{44}O_{22}$ requires: C, 56.01; H, 4.92%].

3-Hydroxymorindone-6-β-primeveroside nonaacetate (**30**), pale yellow needles, mp 263–265°; UV λ_{max}^{MeOH} nm (log ε): 263.5 (4.66), 282 (4.31), 339 (3.79); IR ν_{max}^{KBr} cm^{-1}: 1740, 1665, 1585. [Found: C, 54.49; H, 5.11. $C_{42}H_{46}O_{24} \cdot 1/2H_2O$ requires: C, 54.61; H, 4.89%].

5,6-Dihydroxylucidin-3-β-primeveroside decaacetate (**32**), mp 251–253°; UV λ_{max}^{MeOH} nm (log ε): 272 (4.80), 330 (3.93); IR ν_{max}^{KBr} cm^{-1}: 1750, 1670, 1590. [Found: C, 54.64; H, 4.52. $C_{46}H_{48}O_{26}$ requires: C, 54.34; H, 4.76%].

Acid hydrolysis of anthraquinone glycosides. **11** (10 mg) was hydrolysed with 10% HCl (10 ml) at 85–90° for 2.5 hr. After cooling, the reaction was extracted with Et_2O (15 ml × 5). The Et_2O layer was dried and concd to give a residue (5 mg), which on recrystallization from MeOH gave red needles (3 mg). This substance was identical with **7** (IR, NMR, MS). The aq. layer was neutralized with Amberlite IR-410 (OH^- form) and evapd to dryness. The residue after trimethylsilylation, was subjected to GLC, which showed the presence of glucose and xylose. **12** (10 mg) and **13** (7 mg) were hydrolysed in the same way as above. They gave **6** (2.0 mg) and **8** (1.2 mg) as aglycones, and glucose and xylose as sugars, respectively. In each case, an aliquot of the aq. layer was also examined by D-glucose oxidase [11]. The results indicated that the glucose obtained belongs to D-series.

Photolysis of anthraquinone glycosides. **11** (2 mg) was irradiated in H_2O (200 ml) with a high pressure Hg lamp for 8 hr. Evapn of the solvent *in vacuo* gave a residue, which indicated on GLC after trimethylsilylation the presence of primeverose as a sole sugar. Hydrolysis of **12** (2 mg), **13** (2 mg) and the dimeric compound (1 mg) in the same way also revealed the presence of primeverose, respectively.

Synthesis of 3-*hydroxymorindone* (**7**) *and* 2,6-*dimethyl*-1,3,5,7-*tetrahydroxyanthraquinone* (**33**). Boric anhydride (0.3 g) was added to a suspension of 3,5-dihydroxy-*p*-toluic acid (0.3 g) and protocatechuic acid (0.3 g) in conc H_2SO_4 (5.0 g) and the mixture was heated at 120–130° with stirring for 20 min. After being cooled, the reaction was poured into ice-H_2O and extracted with EtOAc (200 ml × 5). The EtOAc extract was washed with H_2O, dried and concd *in vacuo*. The residue was subjected to DCCC-A (52 glass tubes) and 7 ml fractions were collected. Fr. 201–250 were combined and concd *in vacuo*. The residue (11.5 mg) was recrystallized from MeOH yielding red needles of 3-hydroxymorindone (7.5 mg), identical with the quinone (**7**) isolated from the cell cultures (IR, NMR, MS). **33** was obtained from the residue (50.5 mg) of the combined fr. 105–190 after recrystallization from MeOH as orange needles (44.2 mg), mp > 300°. UV λ_{max}^{MeOH} nm (log ε): 288 (4.62), 332 (4.09), 418.5 (4.05); IR ν_{max}^{KBr} cm^{-1}: 3370, 1570. [Found: C, 63.88; H, 4.01. Calc. for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03%]. It (10 mg) was acetylated in the usual way and the product was recrystallized from MeOH to give 2,6-dimethyl-1,3,5,7-tetraacetoxyanthraquinone (**21**) as pale yellow needles, mp 278–279°. UV λ_{max}^{MeOH} nm (log ε): 263 (4.51), 280 (sh) (4.21), 339 (3.75); IR ν_{max}^{KBr} cm^{-1}: 1760, 1675, 1590. [Found: C, 61.43; H, 4.26. $C_{24}H_{20}O_{10}$ requires: C, 61.55; H, 4.26%].

Synthesis of 5,6-*dihydroxylucidin* (**8**). A mixture of 3-hydroxymorindone tetraacetate (**29**) (10 ml), NBS (10 mg) and benzoyl peroxide (1 mg) in CCl_4 (10 mg) was refluxed for 24 hr. After evapn of the solvent, the resulting residue was washed with hot H_2O, dried and recrystallized from HOAc to give pale yellow needles of 2-bromomethyl-1,3,4,6-tetraacetoxyanthraquinone, mp 231–232°. UV λ_{max}^{MeOH} nm (log ε): 259 (4.23), 277(sh) (3.84), 339 (3.47); IR ν_{max}^{KBr} cm^{-1}: 1765, 1675, 1590; ^{1}H NMR: δ 2.34, 2.42, 2.45, 2.54 (each 3H, *s*, 4 × ArOAc), 4.42 (2H, *s*, benzyl —CH_2Br), 7.57 (1H, *d*, J = 8.5 Hz, H-7), 7.97 (1H, *s*, H-4), 8.18 (1H, *d*, J = 8.5 Hz, H-8). [Found: C, 61.17; H, 3.49; Br, 17.88. $C_{23}H_{16}O_{10}Br$ requires: C, 61.07; H, 3.56; Br, 17.67%]. This substance (10 mg) was refluxed with freshly fused NaOAc (10 mg) in Ac_2O (5 ml) for 1 hr. The reaction was cooled and poured into ice-H_2O. The ppt. was washed with H_2O, dried and recrystallized from MeOH to give yellow needles of 5,6-dihydroxylucidin pentaacetate (**31**), mp 222–224°. UV λ_{max}^{MeOH} nm (log ε): 259 (4.55), 277(sh) (4.09), 334 (3.75); IR ν_{max}^{KBr} cm^{-1}: 1770, 1670, 1585. [Found: C, 58.44; H, 3.86. $C_{25}H_{20}O_{12}$ requires: C, 58.61; H, 3.93%]. Conc H_2SO_4 (0.5 ml) was added to a soln of this substance (8 mg) in MeOH (5 ml). After being refluxed for 30 min, the reaction was diluted with H_2O. The resulting red ppt. was washed with H_2O, dried and recrystallized from MeOH to give red needles (mp > 300°). This product was identical with 5,6-dihydroxylucidin (**8**) isolated from the cell cultures (IR and NMR).

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REFERENCES

1. Thomson, R. H. (1971) *Naturally Occurring Quinones*, 2nd edn, p. 367. Academic Press, London.
2. Zenk, M. H., El-Shagi, H. and Schulte, U. (1975) *Planta Med.* (Suppl) 79.
3. Leistner, E. (1975) *Planta Med.* (Suppl.) 214.
4. Ogihara, Y., Inoue, O., Otsuka, H., Kawai, K., Tanimura, T. and Shibata, S. (1976) *J. Chromatogr.* **128**, 218.

5. Jacobson, R. A. and Adams, R. (1925) *J. Am. Chem. Soc.* **47,** 283.
6. Inoue, K., Shiobara, Y., Nayeshiro, H., Inouye, H. and Zenk, M. H. (1979) *J. Chem. Soc. Chem. Commun.* 957 and refs. cited therein.
7. Gamborg, O. L., Miller, R. A. and Ojima, K. (1968) *Exp. Cell Res.* **50,** 151.
8. Stikhin, V. A., Ban'kovskii, A. I. and Perel'son, M. E. (1968) *Khim. Prir. Soedin.* **4,** 273.
9. Murti, V. V. S., Seshadri, T. R. and Sivakumaran, S. (1970) *Indian J. Chem.* **8,** 779.
10. Vermes, B., Farkas, L. and Wagner, H. (1980) *Phytochemistry* **19,** 119.
11. Dahlqvist, A. (1961) *Biochem. J.* **80,** 547.