Phytochemistry, Vol. 20, No. 7, pp. 1701–1705, 1981.
Printed in Great Britain.

0031-9422/81/071701-05 $02.00/0

TWO QUINONES FROM CALLUS CULTURES OF *ECHIUM LYCOPSIS**

HIROYUKI INOUYE, HARUKI MATSUMURA, MASAKO KAWASAKI, KENICHIRO INOUE, MINORU TSUKADA and MAMORU TABATA

Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan

(*Received* 26 *September* 1980)

Key Word Index—*Echium lycopsis*; Boraginaceae; plant tissue culture; quinones; echinone; echinofuran.

Abstract—Two new quinones, echinone and echinofuran, have been isolated from callus cultures of *Echium lycopsis* along with several acyl esters of shikonin. The structures of both quinones have been established by spectroscopic methods and by chemical degradations.

INTRODUCTION

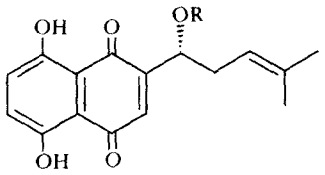
The biosynthesis of shikonin, the red naphthoquinone pigment of the root of *Lithospermum erythrorhizon* Sieb. et Zucc. (Boraginaceae), has recently been investigated both biologically and chemically by using the tissue culture system [1–3]. In the present study on the related compounds produced by the callus cultures of another boraginaceous plant, *Echium lycopsis*, two kinds of new quinones have been isolated, in addition to known fatty acid esters of shikonin (**1**). The determination of the chemical structures of these quinones, echinone (**2**) and echinofuran (**3**), is reported in this paper.

RESULTS AND DISCUSSION

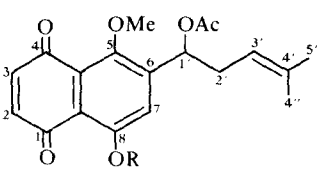
Echinone (**2**), $C_{19}H_{20}O_6$, $[\alpha]_D$ −197.9° ($CHCl_3$) was obtained as an orange-coloured oil exhibiting UV absorption maxima (EtOH) at 215, 254, 330 and 440 nm (log ε 4.72, 4.27, 3.35 and 3.85), and IR bands ($CHCl_3$) at 1725, 1655, 1630 and 1600 cm^{-1}. Its ^1H NMR spectrum contained broad singlets (δ 1.58 and 1.70) due to gem methyl groups on a double bond, a singlet (δ 2.13) due to an acetoxyl group, a double doublet (δ 2.50, J = 7.0 and 6.0 Hz) for allyl methylene protons, a deformed triplet (δ 5.13, J = 7.0 Hz) for a vinyl proton, and a double doublet (δ 6.05, J = 6.0 and 7.0 Hz) which was assigned to the proton on the acetoxyl-bearing carbon. These signals closely resembled those of the side chain of acetylshikonin (**4**). Furthermore, the ^1H NMR spectrum of **2** exhibited singlets due to a phenolic methoxyl group (δ 3.93), two quinonoid protons (δ 6.88), one benzene proton (δ 7.28), and a proton of a hydrogen-bonded phenolic hydroxyl group (δ 12.52). The data presented above suggested that echinone (**2**) was a 1,4-naphthoquinone having one hydroxyl group at the *peri*-position and a benzene ring that was substituted by a hydroxyl group, a methoxyl group and an alkyl side chain. To elucidate the substitution pattern in the benzene ring, NOE experiments with **2** and its ethyl ether (**5**), $C_{21}H_{24}O_6$, obtained by treating **2** with Ag_2O and ethyl iodide, were carried out. About 10% NOE was observed between the methylene protons (δ 4.17, 2H, q, J = 7.0 Hz) of the ethoxyl group of **5** and the benzene proton (δ 7.27), whereas no NOE was detected between the methoxyl group (δ 3.90, s) and the benzene proton of **2**. This meant that the benzene proton was *ortho* to the hydroxyl group. On the basis of these facts, echinone was assigned structure **2** in which the configuration of the acetoxyl group was not specified, and the ethyl ether was assigned structure **5**. The proposed structure for echinone (**2**) corresponds to a methyl ether of a tautomeric isomer of acetylshikonin (**4**) or its enantiomer acetylalkannin (**6**).

Confirmation of the structure of **2** and the determination of the absolute configuration of the acetoxyl group was carried out by chemically correlating **2** with **1** or alkannin (**7**). (Recently the analysis of the ^1H NMR spectrum of **7** has suggested that it exists in the form of its tautomer. However, the structure of the general type is shown here, cf. [4].) The treatment of **2** with $AlCl_3$ caused demethylation, deacetylation and ring closure to yield compound **8**, $C_{16}H_{10}O_5$, red needles, mp 83–84°, Cotton effect $[\theta]_{341}$ +200 (MeOH). **8** exhibited UV absorption maxima (EtOH) at 216, 280, 486, 515 and 555 nm (log ε 4.52, 3.74, 3.66, 3.73 and 3.65) and IR bands ($CHCl_3$) at 1600, 1560, 1445 and 1265 cm^{-1}. Furthermore, the ^1H NMR spectrum of **8** contained a singlet (δ 1.37) due to two tertiary methyl groups, multiplets (δ 1.56–2.80) due to two methylene groups, a deformed triplet (δ 5.11, J = 7.0 Hz) for the proton on the carbon bearing an ether oxygen, a singlet (δ 7.14) for three aromatic protons, and a singlet (δ 12.44) for two hydrogen-bonded phenolic hydroxyl groups. Reductive acetylation of **8** with Zn–Ac_2O–pyridine gave a leucoacetate (**9**), $C_{24}H_{26}O_9$, mp 222–224°. In the ^1H NMR spectrum of **9**, in addition to the signals for the side chain of **8** and four acetoxyl groups, the signals due to the quinonoid and benzenoid protons, which overlapped with the signal due to $CHCl_3$ in the ^1H NMR of **8**, appeared at δ 7.00 and 7.30 as new benzenoid protons. These observations suggested that, except for the stereochemistry, **8** was the same compound as the alkaline hydrolysis product of the dimethylacrylic ester of

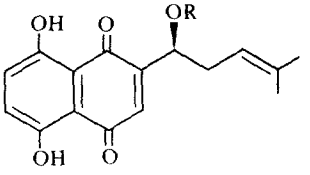
* Part 14 in the series "Quinones and Related Compounds in Higher Plants". For Part 13 see Inoue, K., Shiobara, Y., Ookawa, S., Inouye, H., Taga, T., Yoshida, K. and Osaki, K. (1981) *Chem. Pharm. Bull.* (*Tokyo*) **29**, 558.



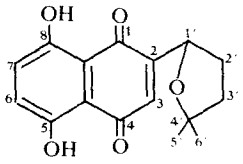
1 R = H
4 R = Ac



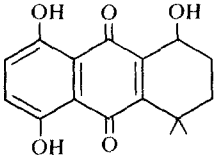
2 R = H
5 R = Et



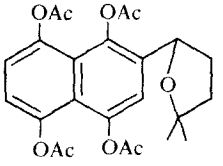
6 R = Ac
7 R = H



8



8a



9

hydroxyalkannin isolated from *Arnebia nobilis* Rachinger [5]. On the other hand, **8** appeared to be identical with 'cycloshikonin' derived from shikonin (**1**) by Brockmann who assigned it structure **8a** [6]. $AlCl_3$ treatment of optically pure acetylshikonin (**4**) [mp 108.5–109, Cotton effect: $[\theta]_{352}$ +6240 (MeOH)] in the same way as in the case of echinone (**2**) gave a substance that had identical UV, IR and ^1H NMR spectra to the compound (**8**) obtained from **2**. The newly obtained product, however, gave a high value for the Cotton effect $[[\theta]_{341}$ +6140 (MeOH)], a mp of 87–88.5° and no depression on mmp with **8** (formed from **2**). The same treatment of alkannin (**7**) (isolated from the rhizomes of *Macrotomia euchroma*) with slightly lower optical purity [Cotton effect: $[\theta]_{357}$ −5070 (MeOH)] yielded the same product showing a Cotton effect of opposite sign $[[\theta]_{341}$ −4420 (MeOH)] and a mp of 82–83.5°. Therefore, it was concluded that the structure of cycloshikonin proposed by Brockmann as **8a** should be corrected to **8***, and that the compound **8** derived from echinone (**2**), and, therefore, echinone itself, consists of both enantiomers, although the (1′-*R*)-enantiomer was slightly dominant†.

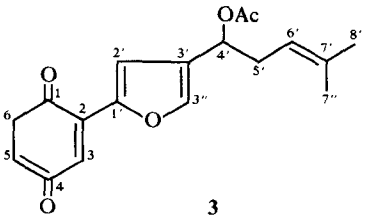
Echinofuran (**3**), $C_{18}H_{18}O_5$, $[\alpha]_D$ −40.0° ($CHCl_3$), was isolated from the callus extract as an orange-coloured oil, exhibiting UV absorption maxima (EtOH) at 258, 323 and 435 nm (log ε 4.23, 3.52 and 3.60) as well as IR bands ($CHCl_3$) at 1715, 1642, 1585 and 1560 cm^{-1}. Its ^1H NMR spectrum exhibited broad singlets (δ 1.62 and 1.70) due to gem methyl groups on a double bond, a singlet (δ 2.05) due to an acetoxyl group, a triplet (δ 2.56, J = 7.0 Hz) due to allyl methylene protons, a broad triplet (δ 5.07, J = 7.0 Hz) for the vinyl proton and a triplet (δ 5.75, J = 7.0 Hz) which was assigned to the proton on the acetoxyl-bearing carbon. Furthermore, the ^1H NMR spectrum contained in the aromatic region typical sharp singlets of *ortho*-positioned quinonoid protons (δ 6.74 and 6.76) and three broad singlets (δ 7.03, 7.46 and 7.53). The signals given by the protons in the side chain were similar to those given by the side chains of acetylshikonin (**4**) and **2**, and showed that these compounds had the same side chain.

To clarify the structure of the quinone moiety, **3** was converted into a leucoacetate (**10**) by reductive acetylation with Zn–Ac_2O–pyridine. The ^1H NMR spectrum of **10** showed, in the aromatic proton region, a typical ABX pattern (δ 7.02, *dd*, J = 2.5 and 9.0 Hz; δ 7.11, *d*, J = 9.0 Hz; δ 7.51, *d*, J = 2.5 Hz) as well as two broad singlets (δ 6.70 and 7.42). The ABX pattern suggested that **9** was a hydroquinone diacetate with an alkyl substituent and, therefore, that **3** was a monosubstituted benzoquinone.

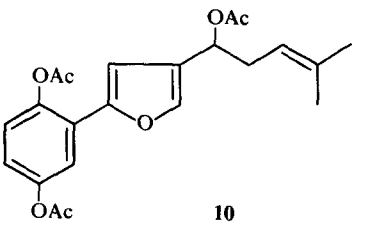
The next problem was to elucidate the structure of the remaining part (C_4H_2O) of the molecule other than the quinone nucleus and the side chain. Echinofuran gave a positive Ehrlich's test, indicating the presence of a furan ring. This was supported by the composition of **3** and the ^1H NMR spectrum of **10**, in which two singlets assignable to the α- and β-protons of the furan ring appeared at δ 7.42 and 6.70, respectively. In the ^1H NMR spectrum of **3**, however, the latter signal appeared at about δ 7.5, which was in accord with the corresponding proton signals of 2-(2-furyl)-benzoquinone-type compounds [6]. The substitution pattern of the furan ring was corroborated by the following method. Compound **3** afforded on work-up with alkaline-H_2O_2 an oxidation product, which was then treated with CH_2N_2 to give a methyl ester (**11**): colourless oil, $[\alpha]_D$ −20.4° ($CHCl_3$). The UV spectrum (MeOH) of **11** had absorption maxima at 220, 256 and 310 nm (log ε 3.74, 4.01 and 3.04), and the IR spectrum ($CHCl_3$) had bands at 3400, 1710 and 1590 cm^{-1}. The ^1H NMR spectrum exhibited singlets of a carbomethoxyl group at δ 3.87 and those of furanoid protons at δ 7.13 and 7.47 in addition to signals originating from the side chain. All

* The same conclusion has also been reached regarding the structure of cycloshikonin by Prof. U. Sankawa of the Faculty of Pharmaceutical Sciences, Tokyo University, who used X-ray analysis (personal communication).

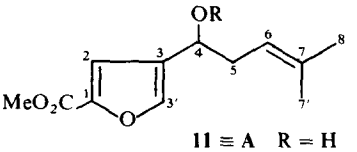
† It has been found recently that the callus tissues of *Echium lycopsis* produce not only acylshikonin but also their corresponding acylalkannins, some of which are present in greater quantities (Tabata, M., Tsukada, M., Arao, N. and Fukui, H., unpublished data).



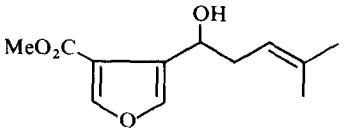
3



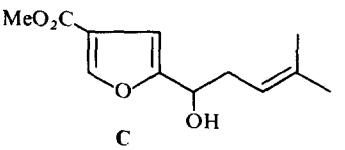
10



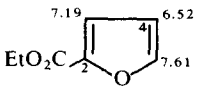
11 ≡ **A** R = H
12 R = COC_6H_4Br-(*p*)



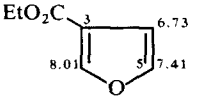
B



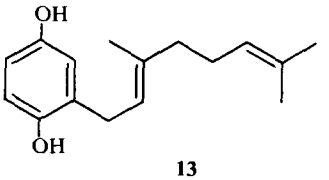
C



D



E



13

these spectral data suggested that **11** was a derivative of furoic acid methyl ester. Further, the appearance of both proton signals as singlets suggested that **11** had one of the three possible structures **A**, **B** or **C**. Since the chemical shifts for the two protons of the furan ring of **11** agree with those for the protons on C-3 and C-5 of ethyl 2-furoate (**D**), but not with those for the protons on C-2 and C-4 or the protons on C-2 and C-5 of ethyl 3-furoate (**E**), the structure of **11** was concluded to be **A** [6]. Thus, echinofuran must have the structure shown by formula **3**.

Finally, as regards the stereochemistry of **2**, it was deduced from the application of the benzoate sector rule that the (4′-*S*)-enantiomer is dominant, since the *p*-bromobenzoate (**12**) of **11** showed a positive first Cotton effect [7].

Of the two quinone compounds characterized in the present study, echinone (**2**) appears to be a compound belonging to the shikonin–alkannin group, whereas echinofuran (**3**) may be seen as a metabolite arising from the oxidation and the subsequent furan ring formation of the side chain of geranylhydroquinone (**13**), which has already been proved to be a key intermediate in the biosynthesis of shikonin [2].

EXPERIMENTAL

All mps are uncorr. ^{1}H NMR spectra were measured in $CDCl_3$ with TMS as int. standard. TLC and prep. TLC were carried out on Si gel GF_{254} and Si gel PF_{254}, respectively. The spots and bands were visualized either by UV radiation (254 nm) or by exposure to I_2.

Isolation of echinone (**2**), *echinofuran* (**3**) *and acyl derivatives of shikonin.* Fresh callus tissues (wet wt 375 g) of *Echium lycopsis* L., harvested after culturing on Linsmaier and Skoog's basal agar medium containing IAA (1 μM) and kinetin (10 μM) at 25° in the dark for a month, were homogenized in a mortar with $CHCl_3$. The insoluble material was filtered off and the filtrate washed with H_2O, dried and concd *in vacuo*. The residue (703.7 mg) was subjected to prep. TLC ($CHCl_3$). A band at about R_f 0.52 was scraped off and extracted with $CHCl_3$. The $CHCl_3$ extract was concd *in vacuo* to give a mixture of β,β-dimethylacryl-, isobutyl- and isovalerylshikonin (12.2 mg) as a red oil. On alkaline hydrolysis, this mixture afforded shikonin and three fatty acids which were identical (GLC) with authentic β,β-dimethylacrylic, isobutyric and isovaleric acid. A band at about R_f 0.47 was also extracted with $CHCl_3$ and recrystallized from *n*-hexane to give **4** (90.6 mg) as red needles, mp 103.5–105°, Cotton effect (7.57×10^{-4} M, MeOH): $[\theta]_{352}$ +5510. **4** was shown to be identical with an authentic sample (mmp, UV, IR and ^{1}H NMR). The $CHCl_3$ extract from a band at about R_f 0.40 was further purified by prep. TLC (C_6H_6–EtOAc, 9:1; R_f 0.65) to give **3** (36.6 mg) as an orange-coloured oil. $[\alpha]_D^{25}$ −40.0° (*c* 1.14, $CHCl_3$); ^{1}H NMR: δ 1.62 and 1.70 (each 3 H, *s* (*br*), 7″- and 8′-H), 2.05 (3 H, *s*, OAc), 2.56 (2 H, *t*, J = 7.0 Hz, 5′-H), 5.07 (1 H, *t* (*br*), J = 7.0 Hz, 6′-H), 5.75 (1 H, *t*, J = 7.0 Hz, 4′-H), 6.74 (1 H, *s*, 5- or 6-H), 6.76

(1 H, s, 6- or 5-H), 7.03 (1 H, s (br), 3-H), 7.46 (1 H, s (br), 2'- or 3''-H) and 7.53 (1 H, s (br), 3''- or 2'-H). [Found: M = 314.1153, $C_{18}H_{18}O_5$ requires: M = 314.1154]. On Ehrlich test, **3** showed colours changing from orange to green on heating in a boiling water bath. The $CHCl_3$ extract from a band at about R_f 0.34 was also purified by prep. TLC (C_6H_6–EtOAc, 9:1; R_f 0.52) to give **2** (24.5 mg) as a red-orange oil. $[\alpha]_D^{25}$ −197.9° (*c* 1.90, $CHCl_3$); ^{1}H NMR: δ 1.58 and 1.70 (each 3 H, s (br), 4''- and 5'-H), 2.13 (3 H, s, OAc), 2.50 (2 H, dd, J = 6.0 and 7.0 Hz, 2'-H), 3.93 (3 H, s, OMe), 5.13 (1 H, t, J = 7.0 Hz, 3'-H), 6.05 (1 H, dd, J = 6.0 and 7.0 Hz, 1'-H), 6.88 (2 H, s, 2- and 3-H), 7.28 (1 H, s, 7-H) and 12.52 (1 H, s, OH, disappeared by D_2O). [Found: M = 344.1251, $C_{19}H_{20}O_6$ requires: M = 344.1259]. The $CHCl_3$ extract from a band at about R_f 0.18 afforded β-hydroxyisovalerylshikonin (53.9 mg) as red needles, which was identical with an authentic sample (UV, IR and ^{1}H NMR).

Ethylation of **2**. To a soln of **2** (20 mg) in $CHCl_3$ (6 ml) was added EtI (1.6 ml) and Ag_2O (20 mg), and the mixture was stirred at room temp. overnight. The resulting Ag salt was filtered off and the filtrate concd *in vacuo*. The residue was purified by prep. TLC (C_6H_6–EtOAc, 9:1; R_f 0.20) to give **5** (14.5 mg) as a yellow oil. UV λ_{max}^{EtOH} nm (log ε): 250 (4.20), 318 (3.18) and 414 (3.68); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1655, 1580, 1370, 1330 and 1220; ^{1}H NMR: δ 1.51 (3 H, t, J = 7.0 Hz, $OCH_2C\underline{H}_3$), 1.51 and 1.68 (each 3 H, s, (br) 4''- and 5'-H), 2.12 (3 H, s, OAc), 2.52 (2 H, t (br), J = 7.0 Hz, 2'-H), 3.90 (3 H, s, OMe), 4.17 (2 H, q, J = 7.0 Hz, $OC\underline{H}_2Me$), 5.10 (1 H, deformed t, J = 8.0 Hz, 3'-H), 6.13 (1 H, t, J = 8.0 Hz, 1'-H), 6.77 (2 H, s, 2- and 3-H), 7.27 (1 H, s, 7-H). [Found: M = 372.1554, $C_{21}H_{24}O_6$ requires: M = 372.1573].

Treatment of **2** *with* $AlCl_3$. To a soln of **2** (14.0 mg) in nitrobenzene (1 ml) was added a soln of $AlCl_3$ (50 mg) in nitrobenzene (2 ml) and the mixture was stirred at room temp. for 1.5 hr. Ice-H_2O and conc HCl (5 ml) were added successively to the reaction. The resulting violet H_2O layer, after washing with Et_2O (10 ml × 3) and adding conc HCl (5 ml), was warmed at 70° for 1 hr, cooled with ice and extracted with Et_2O (20 ml × 3). The Et_2O extract was washed with H_2O, dried and concd. The residue was purified by prep. TLC (C_6H_6–EtOAc, 4:1; R_f 0.60) to give **8** (6.7 mg) as red needles, mp 83–84°, CD (4.20×10^{-4} M, MeOH): $[\theta]_{289}$ 0, $[\theta]_{341}$ +200, $[\theta]_{380}$ 0, $[\theta]_{417}$ −170, $[\theta]_{466}$ −50, $[\theta]_{506}$ 0; ^{1}H NMR: δ 1.37 (6 H, s (br), 5'- and 6'-H), 1.56–2.80 (4 H, m, 2'- and 3'-H), 5.11 (1 H, deformed t, J = 7.0 Hz, 1'-H), 7.14 (3 H, s, 3-, 6- and 7-H) and 12.44 (2 H, s, OH × 2, disappeared by D_2O). [Found: C, 66.91; H, 5.45. Calc. for $C_{16}H_{16}O_5$: C, 66.66; H, 5.95%].

Reductive acetylation of cycloshikonin (**8**). A mixture of **8** (30 mg), Zn powder (30 mg), Ac_2O (1 ml) and pyridine (1 ml) was allowed to stand at room temp. for 1 hr. Ice-H_2O was poured into the mixture and the whole was extracted with Et_2O (10 ml × 3). The Et_2O layer was washed with N HCl, H_2O, 5% $NaHCO_3$, H_2O and the residue was recrystallized from MeOH to give **9** (31 mg) as colourless needles, mp 222–224°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1610, 1360, 1185 and 1015; ^{1}H NMR: δ 1.28 (3 H, s (br), 5'- or 6'-H), 1.32 (3 H, s (br), 6'- or 5'-H), 1.67–2.17 (4 H, m, 2'- and 3'-H), 2.30 (12 H, s, 4 × OAc), 5.10 (1 H, t (br), J = 7.0 Hz, 1'-H), 7.00 (2 H, s, 6- and 7-H), 7.30 (1 H, s, 3-H). [Found: C, 62.85; H, 5.99. Calc. for $C_{24}H_{26}O_9$: C, 62.88; H, 5.72%].

Treatment of acetylshikonin (**4**) *and alkannin* (**7**) *with* $AlCl_3$. $AlCl_3$ (500 mg) was added to a soln of **4** (mp 108.5–109°, CD (7.57×10^{-4} M, MeOH): $[\theta]_{284}$ 0, $[\theta]_{306}$ −3340, $[\theta]_{323}$ 0, $[\theta]_{352}$ +6240, $[\theta]_{408}$ +1000, $[\theta]_{430}$ +610, $[\theta]_{441}$ 0, $[\theta]_{467}$ −2340, $[\theta]_{567}$ 0, $[\theta]_{572}$ +390) (50 mg) in nitrobenzene (15 ml) and the mixture was treated in the same way as above. The reaction product was recrystallized from petrol and C_6H_6 to give **8** (34 mg) as red needles, mp 87–88.5°. CD (1.63×10^{-3} M, MeOH): $[\theta]_{300}$ 0, $[\theta]_{321}$ +5620, $[\theta]_{341}$ +6140, $[\theta]_{389}$ 0, $[\theta]_{412}$ −2000, $[\theta]_{466}$ −1330, $[\theta]_{567}$ 0, $[\theta]_{571}$ +210. This substance was identical with a sample of **8** derived from **2** (mmp, UV, IR and ^{1}H NMR). **7** (mp 144.5–146°, CD (1.98×10^{-3} M, MeOH): $[\theta]_{305}$ 0, $[\theta]_{315}$ +1000, $[\theta]_{324}$ 0, $[\theta]_{357}$ −5070, $[\theta]_{466}$ −1240) gave on the same treatment needles, mp 82–83.5°. CD (2.42×10^{-3} M, MeOH): $[\theta]_{321}$ −4020, $[\theta]_{341}$ −4420, $[\theta]_{389}$ 0, $[\theta]_{409}$ +1200, $[\theta]_{466}$ −1080. This substance was also identical with a sample of **8** derived from **2** (UV, IR and ^{1}H NMR).

Reductive acetylation of echinofuran (**3**). A mixture of **3** (11 mg), Zn powder (15 mg), Ac_2O (0.5 ml) and pyridine (0.5 mg) was treated the same as for the reductive acetylation of **8** and the crude product was purified by prep. TLC (C_6H_6–EtOAc, 9:1; R_f 0.42) to give **10** (8.2 mg) as a colourless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1755, 1730, 1375, 1240 and 1170; ^{1}H NMR: δ 1.62 and 1.69 (each 3 H, s (br), 7''- and 8'-H), 2.05 (3 H, s, alcoholic OAc), 2.29 and 2.36 (each 3 H, s, phenolic OAc), 2.55 (2 H, t (br), J = 7.0 Hz, 5'-H), 5.07 (1 H, t (br), J = 7.0 Hz, 6'-H), 5.73 (1 H, t, J = 7.0, 4'-H), 6.70 (1 H, s (br), 2'-H), 7.02 (1 H, dd, J = 2.5 and 9.0 Hz, 5-H), 7.11 (1 H, d, J = 9.0 Hz, 6-H), 7.42 (1 H, s (br), 3''-H) and 7.51 (1 H, d, J = 2.5 Hz, 3-H). [Found: M = 400.1522, $C_{22}H_{24}O_7$ requires: M = 400.1510].

H_2O_2 oxidation of **3**. To a soln of **3** (23.1 mg) in MeOH (0.5 mg) was added 30% H_2O_2 (1.5 ml) and 10% NaOH (1.5 ml) under ice-cooling. The mixture was stirred for 1 hr, neutralized with N HCl and extracted with Et_2O (5 ml × 3). The Et_2O extract was washed with H_2O, dried and concd. The residue was again dissolved in Et_2O and methylated with ethereal CH_2N_2 and subjected to prep. TLC (C_6H_6–EtOAc, 4:1; R_f 0.25) to give **11** (13.2 mg) as a colourless oil. ^{1}H NMR: δ 1.62 and 1.71 (each 3 H, s (br), 7'- and 8-H), 2.00 (1 H, s (br), OH, disappeared by D_2O), 2.45 (2 H, t, J = 7.0 Hz, 5-H), 3.87 (3 H, s, $COOC\underline{H}_3$), 4.63 (1 H, t, J = 7.0 Hz, 4-H), 5.11 (1 H, t (br), J = 7.0 Hz, 6-H), 7.13 (1 H, s, 2-H) and 7.47 (1 H, s, 3'-H). [Found: M = 224.1049, $C_{12}H_{16}O_2$ requires: M = 224.1060].

p-Bromobenzoylation of furoic acid methyl ester (**11**). To a soln of **11** (15 mg) in pyridine (1 ml) was added *p*-bromobenzoyl chloride (22 mg) and the mixture was stirred at room temp. for 2 hr. After usual work-up, the crude product was purified by prep. TLC (C_6H_6, R_f 0.45) and recrystallized from MeOH to give **12** (19.8 mg) as colourless needles, mp 48–50°. UV λ_{max}^{MeOH} nm (log ε): 248 (3.55) and 282 (2.13); CD (1.0927×10^{-2} M, MeOH): $[\theta]_{290}$ 0, $[\theta]_{258.5}$ +7500, $[\theta]_{245}$ 0, $[\theta]_{235}$ −2700, $[\theta]_{217}$ −1440 and $[\theta]_{210}$ 0; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1700, 1583, 1430, 1260 and 1095; ^{1}H NMR: δ 1.63 and 1.68 (each 3 H, s (br), 7'- and 8-H), 2.67 (2 H, t, J = 7.0 Hz, 5-H), 3.88 (3 H, s, COOMe), 5.10 (1 H, t (br), J = 7.0 Hz, 6-H), 5.97 (1 H, t, J = 7.0 Hz, 4-H), 7.23 (1 H, s (br), 2-H), 7.58 (1 H, s, 3'-H), 7.48–7.96 (4 H, $A_2'B_2'$ type, 4 × arom. H).

Acknowledgements—We wish to thank Prof. M. H. Zenk, Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität Bochum, for providing us with the seed of *Echium lycopsis*; the staff of the Microanalytical Centre of this University for microanalyses; Dr. T. Shingu of Kobegakuin University and Dr. Y. Kuroda of this Faculty for ^{1}H NMR measurements and Dr. K. Kuriyama of Shionogi Research Laboratory for CD measurements and helpful discussions.

REFERENCES

1. Tabata, M., Mizukami, H., Hiraoka, N. and Konoshima, M. (1974) *Phytochemistry* **13**, 927.
2. Mizukami, H., Konoshima, M. and Tabata, M. (1978) *Phytochemistry* **17**, 95.
3. Inouye, H., Ueda, S., Inoue, K. and Matsumura, H. (1979) *Phytochemistry* **18**, 131.

4. Papageorgiou, V. P. (1979) *Planta Med.* **37**, 185.
5. Shulka, Y. N., Tandon, J. S., Bhakuni, D. S. and Dhar, M. N. (1971) *Phytochemistry* **10**, 1909.
6. Brockmann, H. (1935) *Annalen* **521**, 1.
7. Eugster, C. H. and Bosshard, P. (1963) *Helv. Chim. Acta* **46**, 815.
8. Pouchert, C. J. and Campbell, J. R. (eds.) (1974) *The Aldrich Library of NMR Spectra* Vol. 8, p. 10. Aldrich Chemical Co., Milwaukee, Wisconsin.
9. Harada, N., Ohashi, M. and Nakanishi, K. (1968) *J. Am. Chem. Soc.* **90**, 7349.