corresponding to the gradual loss of up to 3 molecules of water in (**1a**) and up to 4 molecules in (**3a**).

EXPERIMENTAL

Optical rotations were recorded in MeOH. C.d. measurements were done by Mrs. B. Romano in EtOH. I.R. spectra were recorded on KBr pellets. NMR spectra were determined in $CDCl_3$ (or deuteriopyridine, as stated) containing tetramethylsilane as internal standard. TLC was carried on Si gel G (Merck) and spots were developed with iodine vapour. MS were taken under the direction of Dr. Z. Zaretskii. Analyses were performed under the direction of Mr. R. Heller.

Plant material. Physalis peruviana was collected (P.D.S. and S.S.S.) in India, the Ooty Hills, during the summer 1970 and then raised (A.A.) in our nursery at Bet Dagan, Israel, from seeds of the above specimens.

Isolation procedure. Crushed air-dried leaves (1 kg) were exhaustively extracted with MeOH; the extract was concentrated to a vol. of *ca* 2·5 l. a similar vol. of H_2O was added and the mixture was extracted with hexane to remove pigments. The residual sol was re-extracted with Et_2O; the ethereal extract was washed with H_2O, dried and the solvent removed to leave a green residue (*ca* 20 g) which was then chromatographed on Si gel H (1 kg): the column was eluted successively with C_6H_6–EtOAc (7:3) giving **2** (0·1 g), C_6H_6–EtOAc (1:1) giving 2,3-dihydro-**1a** (0·1 g) and **1a** (2 g), and then EtOAc–MeOH (49:1) giving **3a** (2 g). Physalin A (2) mp 263–265° (Me_2CO), withanolide E (**1a**), mp 167–168° (Me_2CO) and 2,3-dihydrowithanolide E, mp 273–275° (Me_2CO) were identified by direct comparison with authentic samples.

4β-Hydroxywithanolide E (**3a**), mp 197–198° (EtOAc). $[\alpha]_D$ +95·8° (*c* 0·5); *c.d.* λ_{max}/nm ($\Delta\epsilon$): 400 (0), 340 (+1·42), 304 (0), 294 (−0·32), 282 (0), 252 (+4·25), 217·5 (+13·91), strongly negative at shorter wavelengths. ν_{max} 1710 and 1670 cm^{-1}, λ_{max}^{EtOH} 215 nm (ϵ 17800) (Found: C, 66·80; H, 7·75; M^+ 502. $C_{28}H_{38}O_8$ requires C, 66·91; H, 7·62; M, 502·58). The acetate (**3b**), prepd. with Ac_2O–C_5H_5N, was purified by preparative TLC using C_6H_6–EtOAc (1:4). The compound could not be induced to crystallize. ν_{max} 1737, 1700 *sh* and 1677 cm^{-1}; λ_{max}^{EtOH} 218 nm (ϵ 17500). (Found: C, 65·92; H, 7·30; *m/e* 526 (M^+ −18). $C_{30}H_{40}O_9$ requires C, 66·16; H, 7·40; M, 544·62).

Acknowledgement—We are grateful to Prof. T. Matsuura for a sample of physalin A.

REFERENCES

1. Lavie, D., Kirson, I., Glotter, E., Rabinovich, D. and Shaked, Z. (1972) *J. Chem. Soc. Chem. Commun.* 877.
2. Bates, R. B. Morehead, S. R. (1974) *J. Chem. Soc. Chem. Commun.* 125.
3. Begley, M., Crombie, L., Ham, P. J. and Whiting, D. A. (1973) *J. Chem. Soc. Chem. Commun.* 821.
4. Abraham, A., Kirson, I., Lavie, D. and Glotter, E. (1975) *Phytochemistry* **14**, 189.
5. For instance, Shoppee, C. W. and Lack, R. E. (1964) *J. Chem. Soc.* 3611.
6. Matsuura, T., Kawai, M., Nakashima, R. and Butsugan, Y. (1970) *J. Chem. Soc.* (*C*) 664.
7. Kirson, I., Glotter, E., Lavie, D. and Abraham, A. (1971) *J. Chem. Soc.* (*C*) 2032.

Phytochemistry, 1976, Vol. 15, pp. 342–343 Pergamon Press. Printed in England

ISOLATION OF 2-(4-HYDROXYBENZYL)MALIC ACID FROM *LYCORIS RADIATA*

TORU KOIZUMI*, YO ISOGAI†, SEIICHIRO NOMOTO‡, KOICHI SHUDO‡ and TOSHIHIKO OKAMOTO‡

*Faculty of Pharmaceutical Sciences, University of Toyama, Gofuku, Toyama 930, Japan; †Department of Biology, College of General Education, University of Tokyo, Komaba, Meguro-ku, Tokyo 153, Japan; ‡Laboratory of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

(*Received* 5 *August* 1975)

Key Word Index—*Lycoris radiata*; Amaryllidaceae; auxin; 2-(4-hydroxybenzyl)-malic acid; phenolic compound.

Plant. Lycoris radiata Herb. is an infertile triploid plant. The clone used started from a single bulb cultivated in the medicinal herb garden of the Faculty of Pharmaceutical Sciences, University of Tokyo, at Kemigawa, Chiba Prefecture. *Previous work.* None on this species. The compound reported was isolated from *Petalostemon gattingeri* Heller, (Leguminosae) as a germination inhibitor of the seeds of *Arenaria patula* Michx. [1].

Plant part examined. L. radiata shows a peculiar growth pattern [2] in early August, scapes with flower buds grow out of the bulb, but without leaves, and elongate very rapidly, reaching a height of 40–50 cm within 10 days. Then the flowers come out, and the whole infloresence dies out before the leaves emerge around November. Scapes of 30–35 cm with buds were used.

Isolation and identification. The MeOH extract of the fresh material was concentrated *in vacuo*. The EtOAc soluble part was separated into acidic and non-acidic fractions. The acidic fraction was chromatographed over a mixture of active charcoal and Celite, and eluted with a mixture of H_2O and Me_2CO. The residue obtained from the 50–60% Me_2CO eluate gave a weak growth promotion in the *Avena* section test. The EtOAc soluble part of the residue was separated by adsorption column chromatography over silicic acid and eluted with C_6H_6–EtOAc with increasing amount of EtOAc. The C_6H_6–EtOAc (2:1) fraction yielded a crystalline product, which was further purified by silicic acid chromatography (C_6H_6–EtOAc). The colorless crystals were recrystallized twice from C_6H_6–MeOH to give needles, mp 178–180°. (*Anal.* Found: C, 56·23; H, 5·05. Calc. for $C_{11}H_{12}O_6 \cdot 1/12$ C_6H_6: C, 56·00; H, 5·07%). MS (*m/e*); 240 (M^+), 222, 204, 150, 132, 131, 107, 94. $[\alpha]_D^{28}$ −19·9° (MeOH). IR ν_{max} (KBr) cm^{-1}: 3480–3100, 1725 (COOH). The 60 MHz NMR spectrum of the compound in $(CD_3)_2CO$ showed the presence of two methylene groups (2H, *s* at

δ2·95, 2H, double doublet at δ2·54 (*J* 16 Hz) and at δ3·03 (*J* 16 Hz)) and four aromatic protons (AA'XX' type quartet at δ6·67–7·15).

The compound formed a dimethyl ester by treatment with CH_2N_2 for 30 min in Et_2O, which was purified by silica gel column chromatography to give a light yellow oil; MS (*m/e*): 268 (Calc. for $C_{13}H_{16}O_6$, M^+), 250, 218, 209, 191, 177, 161, 135, 107, 101, 77, IR ν_{max} (film) cm^{-1}: 3400–3200 (OH), 1750–1730 (COOMe). NMR ($CDCl_3$): δ2·65 (1H, *d*, *J* 16 Hz), 2·92 (2H, *s*), 3·10 (1H, *d*, *J* 16 Hz), 3·69 (3H, *s*, OMe), 3·76 (3H, *s*, OMe), 6·6–7·15 (4H, AA'XX''). Complete methylation with CH_2N_2 in MeOH (24 hr) gave a dimethyl ester monomethyl ether after silica gel column chromatography, as a pale yellow oil; MS (*m/e*): 282 (Calc. for $C_{14}H_{18}O_6$, M^+), 264, 223, 191, 149, 135, 121, 101, 91, 77. NMR ($CDCl_3$): δ2·65 (1H, *d*, *J* 16 Hz), 2·93 (2H, *s*), 3·05 (1H, *d*, *J* 16 Hz), 3·67 3·75, 3·79 (each 3H, *s*, OMe), 6·74–7·20 (4H, AA'XX'). IR ν_{max}(film) cm^{-1}: 3500–3400 (OH). 1740 (COOMe).

From this data the isolated compound was considered to be 2-(4-hydroxy-benzyl)malic acid, and this was confirmed by comparison of IR, NMR, and MS data with those reported by Harris *et al.*[1].

Biological significance. The auxin-like activity was tested with oat coleoptiles (5 mm sections cut 3 mm below the tip), 20 sections per dish floated on 1 ml of the test solutions. The relative mean length of sections in various concentrations of the compound (in parenthesis) to that of the control was: 88% (1000 ppm), 115% (100 ppm), 108% (10 ppm), 105% (1 ppm), and 132% (IAA, 1 ppm).

REFERENCES

1. Harris, T. M., Hay, J. V. and Quarterman, E. (1973) *J. Org. Chem.* **38**, 4457.
2. Isogai, Y., Nomoto, S., Noma, T. and Okamoto T. (1973) *Proc. 8th International Conference on Plant Growth Substances*, p. 9.

Phytochemistry, 1976, Vol. 15, pp 343–344. Pergamon Press. Printed in England.

PHLOROGLUCINOL DERIVATIVES IN *DRYOPTERIS CHRYSOCOMA*

H. S. PURI* and C.-J. WIDÉN

Department of Pharmacognosy, University of Helsinki, SF-00170 Helsinki 17, Finland

and

M. LOUNASMAA

State Institute for Technical Research, Chemical Laboratory, SF-02150 Otaniemi, Finland

(*Received* 21 *June* 1975)

Key Word Index—*Dryopteris chrysocoma*; Polypodiaceae; phloroglucinol derivatives; albaspidin, filixic acid and flavaspidic acid.

In earlier communications, phloroglucinol derivatives of various species of *Dryopteris* from Europe, North America, Africa and Japan have been described [1–5]. The present report on *D. chrysocoma* (Christ) C. Chr., from Himalayas (India), is a part of the same programme. This fern is diploid and has been reported to occur in abundance in some areas of the Himalayas at an altitude of 1·600–2·500 m [6,7]. It is one of the 5 Indian *Dryopteris* species, which are official in the Pharmacopoeia of India (1966) as a source of well known anthelmintic drug male fern [8]. Another fern, *Polystichum squarrosum* (D. Don) Fee, often grows with it. In the present paper, studies on the phloroglucinol derivatives in both of these ferns are reported. Only preliminary chemical work has been done previously on the oleoresin of Indian *Dryopteris* spp. [6,9].

RESULTS AND DISCUSSION

As is evident from the yield of oleo-resin and crude filicin (Table 1), *D. chrysocoma* is quite rich in phloroglucinol derivatives, whereas *P. squarrosum* is totally devoid of them. The phloroglucinol mixture of the crude filicin was separated by column chromatography on Si gel [1–5] and crystals of various homologues of albaspidin, filixic acid and flavaspidic acid were isolated and studied by MS and TLC.

(a) Albaspidin, mp 142–143°. The MS shows 4 molecular peaks at *m/e* 460 (weak), 446 (weak), 432 and 418 corresponding to albaspidins BB, PB, PP and /or AB and AP, respectively.

(b) Albaspidin, mp 133–134°. The MS shows 3 molecular peaks at *m/e* 460, 446 and 432 (weak) corresponding to albaspidins BB, PB and PP, respectively.

(c) Filixic acid, mp 169–170°. The MS shows 4 molecular peaks at *m/e* 668, 654, 640 and 626 (weak), corresponding to filixic acids BBB, PBB, PBP and/or ABH and ABP, respectively.

(d) Flavaspidic acid, mp 150–152°. The MS shows only one molecular peak at *m/e* 446, which corresponds to flavaspidic acid BB. Both *D. chrysocoma* and *D. filix-mas* resemble each other in having considerable amounts of filixic acid and flavaspidic acid. These species, however, differ in that large amounts of albaspidin occur in *D. chrysocoma* and not in *D. filix-mas*. The latter species contains para-aspidin, desaspidin and trisdesaspidin, not found in *D. chrysocoma* (cf. Table). Investigation of the acylfilicinic acids formed by the reductive alkaline clea-

* Present address: Pharmacognosy Research Units, Botany Department Panjab University, Chandigarh 160014, India.