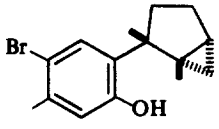
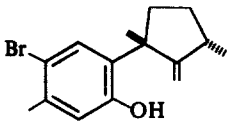
5 mm, 5% Silicone OV-1, isothermal 190°, N_2 at 30 ml/min). 1–4 were identified by comparison of IR, NMR and MS with those reported in the literature.

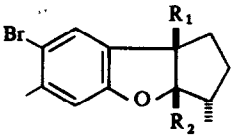
The new compound (5), named aplysinal, gave a positive



(1)



(2)



(3) $R_1 = R_2 = Me$
(4) $R_1 = Me$, $R_2 = CH_2OH$
(5) $R_1 = Me$, $R_2 = CHO$

2,4-DNP test. FTNMR spectrum (100 MHz, $CDCl_3$); 1.05 (3H, *d*, $J = 6$ Hz), 1.30 (3H, *s*), 1.60–1.90 (5H, *m*), (3H, *s*), 6.80 (1H, *s*), 7.16 (1H, *s*), and 9.74 (1H, *s*). MS; *m/e* 308 and 310 (M^+), 279 and 281 ($M^+ - CHO$), 237 and 239 ($M^+ - CHO - CH_2{=}CH{-}Me$), and 200 ($M^+ - CHO - Br$). From these data, structure 5 was deduced for this compound. Finally, 5 was transformed into 4 with $LiAlH_4$. The MS and R_f value (TLC) of the synthetic material were identical to those of natural aplysinol (4).

We have now investigated *Amphiroa zonata* Yendo and *Corallina pilulifera* Postels et Ruprecht collected at Cape Omaezaki, Shizuoka, Japan. We note here that both algae contain all compounds 1–5. It is known that *Laurencia* species produces a large amount of bromosesquiterpenes including 1–4 [2–5]. In the vicinity of Cape Omaezaki, some *Laurencia* species grow; therefore, we cannot rule out the possibility that compounds 1–5 may be derived from these algae. An analysis of local *Laurencia* species and other algae growing around Cape Omaezaki is in progress.

Laurinterol (1) was found to display a marked antibiotic activity against *Staphylococcus aureus* [5]. We have also found both 1 and 2 to have a potent antimicrobial activity against *Bacillus subtilis*.

Acknowledgement—The identification of plant material was kindly carried out by Dr. T. Masaki, Faculty of Fisheries, Hokkaido University, Hakodate, Japan.

REFERENCES

1. Ohta, K. and Takagi, M. (1977) *Phytochemistry* **16**, 1085
2. Irie, T., Suzuki, M., Kurosawa, E. and Masamune, T. (1970) *Tetrahedron* **26**, 3271.
3. Irie, T., Suzuki, M. and Hayakawa, Y. (1969) *Bull. Chem. Soc. Japan* **42**, 843.
4. Sims, J. J., Fenical, W., Wing, R. M. and Radlick, P. (1971) *J. Am. Chem. Soc.* **93**, 3774.
5. Waraszkiewicz, S. M. and Erickson, K. L. (1974) *Tetrahedron Letters* 2003.
6. Yamamura, S. and Hirata, Y. (1963) *Tetrahedron* **19**, 1485.

Phytochemistry, 1977, Vol. 16, pp. 1063–1065. Pergamon Press. Printed in England.

4-HYDROXYDEHYDROMYOPORONE FROM INFECTED *IPOMOEA BATATAS* ROOT TISSUE*

HIROMASA INOUE, NATSUKI KATO and IKUZO URITANI
Department of Agricultural Chemistry, Nagoya University, Chikusa, Nagoya, Aichi 464, Japan

(*Revised received* 7 *January* 1977)

Key Word Index—*Ipomoea batatas*; *Ceratocystis fimbriata*; Convolvulaceae; sweet potato; sesquiterpene; 4-hydroxydehydromyoporone.

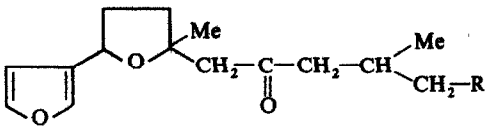
Abstract—A new sesquiterpenoid, 4-hydroxydehydromyoporone, was isolated from *Ceratocystis fimbriata*—infected root tissue of *Ipomoea batatas*. We showed that it was a derivative of myoporane with one hydroxyl group at C-8 and one double bond at C-12 by spectroscopic comparison with known compounds.

INTRODUCTION

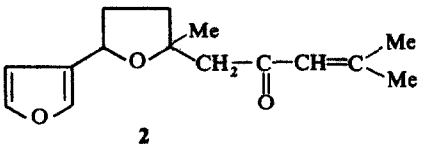
Ipomoea batatas root tissue infected by *Ceratocystis fimbriata* accumulates various sesquiterpenoids such as ipomeamarone (1a) [1, 2], dehydroipomeamarone (2) [3], and ipomeamaronol (1b) [4, 5].

Recently, Wilson's group isolated from diseased sweet potato a new sesquiterpenoid called 4-hydroxymyoporone (3) [6], and we have confirmed that 3 was also accumulated in response to the infection of *C. fimbriata*, by isolating this compound and comparing the structure with that of an authentic sample. We then

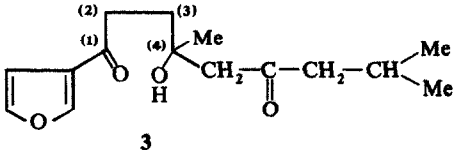
* This paper constitutes Part 129 of the Phytopathological Chemistry of Sweet Potato with Black Rot and Injury. This work was supported in part by a grant from the Ministry of Education.



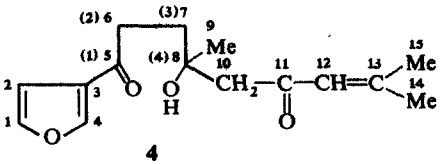
(1a) R = H
(1b) R = OH



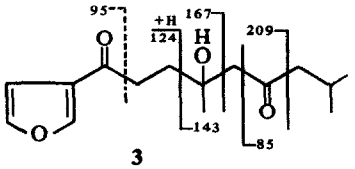
2



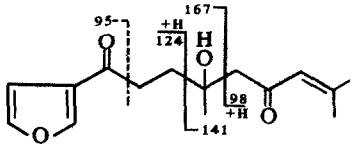
3



4



3



4

Scheme. Related fragments in the MS of 4-hydroxymyoporone (3) and 4-hydroxydehydromyoporone (4).
———: high resolution MS peaks
– – – – –: low resolution MS base peaks

attempted to isolate another compound (4) whose R_f value on TLC was just below that of 3 and which also gave a similar pink colour reaction with Ehrlich's reagent. This paper deals with the structural elucidation of this compound which was assigned as 4.

RESULTS AND DISCUSSION

Infected sweet potato root tissue was extracted with $CHCl_3$–MeOH (1:1) and after addition of H_2O, the $CHCl_3$ layer was evaporated to an oily substance. Si gel column chromatography of this substance yielded a crude fraction containing compound 4. Further purification was accomplished by repeated Kiesel-gel HF_{254} TLC using *n*-hexane–EtOAc (1:1) and C_6H_6–EtOAc (23:2) with HPLC monitoring. The sample used for the analyses was confirmed to be pure by TLC and HPLC.

Table 1. M^+ and important fragment ions in the high resolution MS of 4-hydroxymyoporone (3) and 4-hydroxydehydromyoporone (4)

m/e	Composition	Rel. intensity (3)	(4)
266	$C_{15}H_{22}O_4$	14	—
264	$C_{15}H_{20}O_4$	—	4
251	$C_{14}H_{19}O_4$	6	—
249	$C_{14}H_{17}O_4$	—	6
248	$C_{15}H_{20}O_3$	16	—
246	$C_{15}H_{18}O_3$	—	29
209	$C_{11}H_{13}O_4$	3	—
167	$C_9H_{11}O_3$	37	19
148	$C_9H_8O_2$	100	100
143	$C_8H_{15}O_2$	25	—
141	$C_8H_{13}O_2$	—	29
124	$C_7H_8O_2$	9	6
98	$C_6H_{10}O$	—	13
85	C_5H_9O	53	—

Base peak of the low resolution MS at *m/e* 95 was not shown by high resolution MS. This is perhaps caused by the use of different instruments.

4 showed a similar IR to that of 3 and suggested the presence of furyl, conjugated carbonyl and OH groups, with absence of an unconjugated carbonyl group. The molecular formula $C_{15}H_{20}O_4$ was established by high resolution MS. The fragment ions of 4 and 3 were similar (e.g. $C_9H_{11}O_3$, $C_9H_8O_2$ and $C_7H_8O_2$) but 4 showed ions with *m/e* values 2 amu units less than 3 viz. $C_{14}H_{17}O_4$ to $C_{14}H_{19}O_4$, $C_{15}H_{18}O_3$ to $C_{15}H_{20}O_3$ and $C_8H_{13}O_2$ to $C_8H_{15}O_2$ (Table 1 and Scheme).

The PMR spectrum of 4 was also similar to that of 3 except for a signal at δ6.05 (1H, *m*) assigned to the methine proton at C-12 and signals at δ1.91 (3H,*d*, J = 1.2 Hz) and δ2.17 (3H, *d*, J = 1 Hz) assigned to the gem-di Me group at C-14 and C-15. These differences in the PMR spectra coincide with those found between dehydroipomeamarone (2) and ipomeamarone (1a) [3]. The UV spectrum of 4 showed a maximum at 243 nm (Em = 13600) with shoulders. The value was regarded as the sum of the values at 243 nm of α, β-unsaturated ketone (= 11500 in the case of 2) and of 3-furyl alkanone (= 2400 in the case of 3), and indicated the presence of both α, β-unsaturated ketone and 3-furyl alkanone in the molecule of 4.

All the spectral data indicate that 4 is the correct structure and we have named it 4-hydroxydehydromyoporone.

EXPERIMENTAL

UV spectra were determined in EtOH, and IR spectra as thin films. PMR spectra were run with TMS as the internal standard. HPLC was carried out at a pressure of 10 kg/cm², solvent Et_2O–*n*-hexane (7:3), column 500 × 2.1 mm, packing Hitachi gel 3040 and UV detector (254 nm).

Isolation of compound 4. Sweet potato roots (1.8 kg) were cut vertically into slices 5–10 mm thick, which were inoculated

on the cut surfaces by a spore suspension (*ca* 1×10^7/ml) of *C. fimbriata* Ell. and Halst. and incubated at 29° for 3 days. The infected pieces of the slices were taken and homogenized with *ca* 2 parts of $CHCl_3$–MeOH (1:1) and the homogenate was filtered. The residue was extracted twice with the same solvents. H_2O was added to the combined $CHCl_3$–MeOH soln to adjust the final proportion of $CHCl_3$–MeOH–H_2O to 1:1:1. The $CHCl_3$ layer was evaporated *in vacuo* yielding 46.5 g of an oily substance, which was chromatographed stepwise on a Si gel (350 g) column using 2 l. of *n*-hexane–EtOAc (4:1) and 1 l. of *n*-hexane–EtOAc (1:1). The fraction containing compound **4** (1.1 g) was eluted by the latter solvent just after compound **3**. **4** was isolated by Kiesel gel HF_{254} TLC using *n*-hexane–EtOAc (1:1). Further purification was performed by repeating the TLC in an open system using C_6H_6–EtOAc (23:2). The purity was monitored by HPLC.

UV: λ_{max}^{EtOH} nm (log ε), 243 (4.13); IR: ν_{max}^{neat} (cm^{-1}): 3490 (*br*), 2930 (*s*), 1677 (*br*), 1560 (*br*), 1155 (*s*) and 872 (*s*); PMR: δ_{ppm} ($CDCl_3$): 8.07 (1H, *q*, C-4), 7.43 (1H, *q*, C-1), 6.78 (1H, *q*, C-2), 6.05 (1H, *m*, C-12), 4.45 (1H, *br s*, OH), 2.93 (2H, *m*, C-6), 2.61 (3H, *s*, C-10), 2.17 (3H, *d*, J = 1 Hz, C-15), 1.91 (3H, *d*, J = 1.2 Hz, C-14), 1.85 (2H, *m*, C-7) and 1.24 (3H, *s*, C-9). MS: *m/e* 264.1360 (M^+, $C_{15}H_{20}O_4$ requires 264.1361).

Acknowledgements—We thank Dr. B. J. Wilson for providing the authentic sample of 4-hydroxymyoporone, and express thanks to the members of the Laboratory of Pesticide Chemistry for assistance with PMR, MS and IR measurements and for providing HPLC facilities.

REFERENCES

1. Hiura, M. (1943) *Rep. Gifu. Agr. Coll.* **50**, 1.
2. Kubota, S. and Matsuura, T. (1952) *J. Chem. Soc. Japan* **74**, 101, 197, 248, 668.
3. Oguni, I. and Uritani, I. (1973) *Agr. Biol. Chem.* **37**, 2443.
4. Kato, N., Imaseki, H., Nakashima, N. and Uritani, I. (1971) *Tetrahedron Letters* **13**, 843.
5. Yang, D. T. C., Wilson, B. J. and Harris, T. M. (1971) *Phytochemistry* **10**, 1653.
6. Burka, L. T., Kuhnert, L. and Wilson, B. J. (1974) *Tetrahedron Letters* **46**, 4017.

Phytochemistry, 1977, Vol. 16, pp. 1065–1068. Pergamon Press. Printed in England.

NEUE GERMACROLIDE AUS *CALEA ZACATECHICHI**

FERDINAND BOHLMANN und CHRISTA ZDERO
Institut für Organische Chemie der Technischen Universität Berlin D-1000 Berlin 12, Strasse des 17. Juni 135, Germany

(*Received* 16 *December* 1976)

Key Word Index—*Calea zacatechichi*; Compositae; Heliantheae germacrolides.

Abstract—The aerial parts of *Calea zacatechichi* contain in addition to known acetylenes two new germacrolides, their structures being elucidated by spectroscopic methods. *Calea scabra* contains known acetylenes similar to those of other species and of related genera.

Aus der Gattung *Calea* (Subtribus Galinsoginae, Tribus Heliantheae) sind bereits einige Arten untersucht worden [1, 2]. Bisher wurden hier wie in den Nachbargattungen [2] Dehydrofalcarinon (**11**) und verwandte Verbindungen isoliert. Die in Guatemala heimische *C. zacatechichi* enthält jedoch neben Germacren D (**7**) und Acetylenverbindungen (**8–10**) auch zwei Sesquiterpenlactone mit den Summenformeln $C_{21}H_{26}O_8$ und $C_{19}H_{22}O_7$. Wie aus dem ^1H NMR-Spektrum zu entnehmen ist (s. Tabelle 1.), handelt es sich in beiden Fällen um Methylenlactone, die noch eine weitere Doppelbindung, eine OH-Gruppe und einen Methylacryloyloxy-Rest enthalten. Bei der etwas unpolareren Substanz ist zusätzlich eine *O*-Acetat-Gruppe vorhanden. Die Sauerstoffbilanz ergibt, daß noch eine weitere *O*-Funktion vorhanden sein muß. Das bei 270 MHz voll interpretierbare ^1H NMR-Spektrum der unpolareren Verbindung läßt nach systematischen Entkopplungsexperimenten eindeutig erkennen, daß die Gruppierung A vorliegen muß (s. Tabelle 1.).

* 101. Mitt. in der Serie "Natürlich vorkommende Terpen-Derivate", 100. Mitt. Bohlmann, F., Knoll, K.-H., Zdero, C., Mahanta, P. K., Grenz, M., Suwita, A., Ehlers, D., Le Van, N., Abraham, W.-R. und Natu, A. A. (1977) *Phytochemistry* **16**, 965.