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ECKOLS, NOVEL PHLOROTANNINS WITH A DIBENZO-p-DIOXIN SKELETON POSSESSING INHIBITORY EFFECTS ON α_2-MACROGLOBULIN FROM THE BROWN ALGA ECKLONIA KUROME OKAMURA

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Eckol, 2-phloroeckol and dieckol, novel phlorotannins with a dibenzo-p-dioxin skeleton have been isolated from the brown alga Ecklonia kurome Okamura as plasma α_2-macroglobulin inhibitors and their structures have been determined on the basis of spectral data, in particular, by means of negative NOEs.

The phlorotannins, a class of polyhydroxylated phenols occur in the phaeophyceae[1] and are generally constructed of phloroglucinol moieties linked by aryl-aryl, aryl-ether bonds or of a mixed type.[2] Although the crude extracts show antibacterial activity, all this class of phenols has not been isolated in a natural form due to their instability.[3] In the course of our screening for α_2-macroglobulin inhibitors,[4] the MeOH extract of E. kurome has been found to inhibit the action of α_2-macroglobulin and α_2-plasmin inhibitor which play a role of the suppressing control in the fibrinolytic enzyme system. The bioassay-directed fractionation led to the isolation of new type phlorotannins bearing a dibenzo-p-dioxin skeleton, named eckol (1), 2-phloroeckol (3), and dieckol (4). In this paper, we wish to report the structures of these new compounds.

Eckol (1), mp 243-244 °C, has the following constants: $C_{18}H_{12}O_9$ (m/z 372.0460; calcd. 372.0481); m/z 372(M^+, 100), 264(46), and 232(25); ν_{max}(KBr) 3250(OH) and 1605(aromatic) cm^{-1}; λ_{max}(MeOH) 230(ε 32000) and 290(ε 3100) nm. The ^{13}C NMR spectrum indicated the presence of six non-substituted and twelve O-bearing aromatic carbons. The ^1H NMR spectrum of 1 contained signals characteristic of six aromatic protons, viz. an AB_2 system at δ 5.83(1H, J = 2.2 Hz) and 5.75(2H), an AB system at δ 5.82 and 5.98(J = 2.7 Hz), and a singlet at δ 6.16(1H) as well as six phenolic OH protons at δ 9.17(2H), 9.19, 9.20, 9.48, and 9.53. These NMR spectra are very similar to those of triphloroethol (2)[5] isolated from Laminaria ochroleuca indicating that 1 is composed of three phloroglucinol units. The only difference between the ^1H NMR spectra of 1 and 2 is that the former lacks the signals for one phenolic OH and one aromatic protons suggesting that 1 has an additional aryl-ether linkage. This is supported by the molecular formula ($C_{18}H_{12}O_9$ for 1; $C_{18}H_{14}O_9$ for 2) which indicates one extra unit of unsaturation and the presence of a new oxygen-bearing carbon (δ 122.6) which is characteristic of an aromatic carbon with two oxygenated neighbours. The detailed assignment of all of the proton resonances in the ^1H NMR

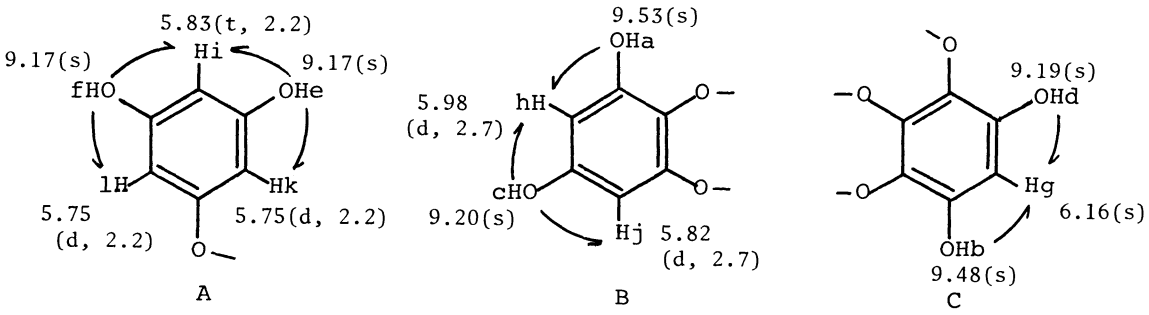


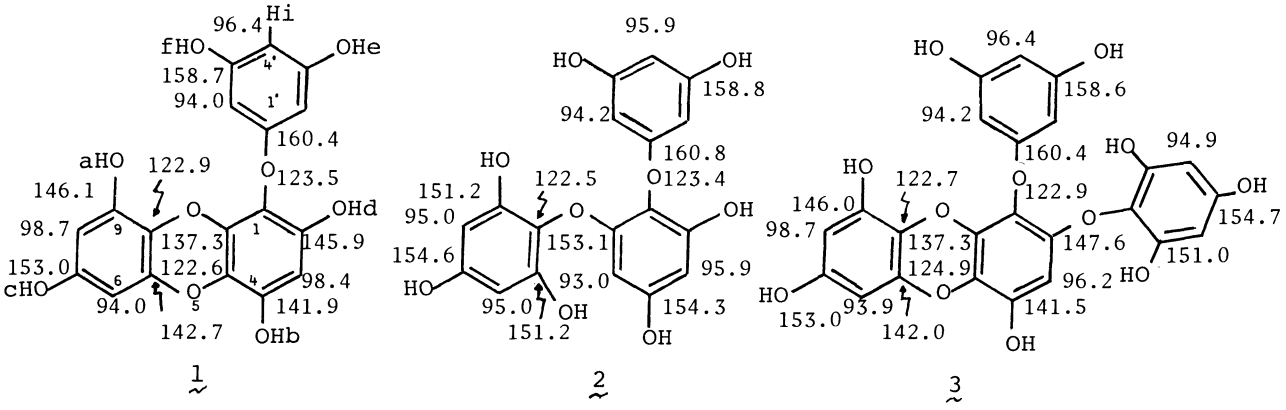
Fig. 1. The partial structures and ^{1}H NMR data (DMSO-d_6) of 1; multiplicity and J values (in Hz) in parentheses, the observed negative NOEs are indicated by arrows.

spectrum was accomplished by means of the negative NOEs (-30—-40%) observed during the course of the NOE experiments.[6] Namely, selective irradiations of the phenolic OH protons Ha, Hb, Hc, Hd, and He,f of 1 led to reductions in the intensities of the aromatic resonances Hh, Hg, Hh + Hj , Hg, and Hk,l + Hi, respectively. Since both Hh and Hj resonances were reduced in intensity upon irradiation of Hc and irradiation of Ha led to a decrease in the intensity of Hh signal, Hh and Hj had to be located ortho to both the OHa and OHc groups, and to the OHc group respectively, suggesting the presence of the partial structure B (Fig. 1). Similarly, the other partial structures A and C were derived from the unambiguous assignment of all the proton signals on the basis of negative NOEs. The linkage of the partial structures A, B, and C led to the structure (1) having a dibenzodioxin skeleton. This tentative structure for eckol was substantiated by the complete assignment of the ^{13}C NMR spectrum shown in Fig. 2 based on $J_{^{13}C-^{1}H}$ values obtained by selective proton decoupling. Thus, the structure (1) was proposed for eckol.[7]

2-Phloroeckol (3), mp 206-207 °C, has the following data: $C_{24}H_{16}O_{12}$ (M^+ 496.0646; calcd. 496.0642); λ_{max}(MeOH) 232(ε 40000) and 292(ε 3400) nm; ν_{max}(KBr) 3250 (OH) and 1615 cm^{-1}. The mass spectrum of 3 showed prominent peaks at m/z 372, 264, and 232 characteristic of eckol, suggesting that 3 is composed of 1 with an additional phloroglucinol moiety. This was supported by the ^{13}C NMR spectrum , summarized in Fig. 2, which contained four extra signals due to the fourth phloroglucinol unit in addition to the eighteen signals corresponding to those of 1. The linkage position of the additional phloroglucinol unit was deduced to be C-2 from the fact that the ^{13}C NMR signals for the basic skeleton in 3 were almost identical with those of 1 except for the signals due to C-2, C-3, and C-4a.[8] Moreover, the negative NOE experiments summarized in Fig. 3 indicated the absence of the phenolic OHd proton found in 1, strongly suggesting that the fourth phloroglucinol unit is linked to C-2 by an aryl-ether bond. Accordingly, 2-phloroeckol has the structure (3).

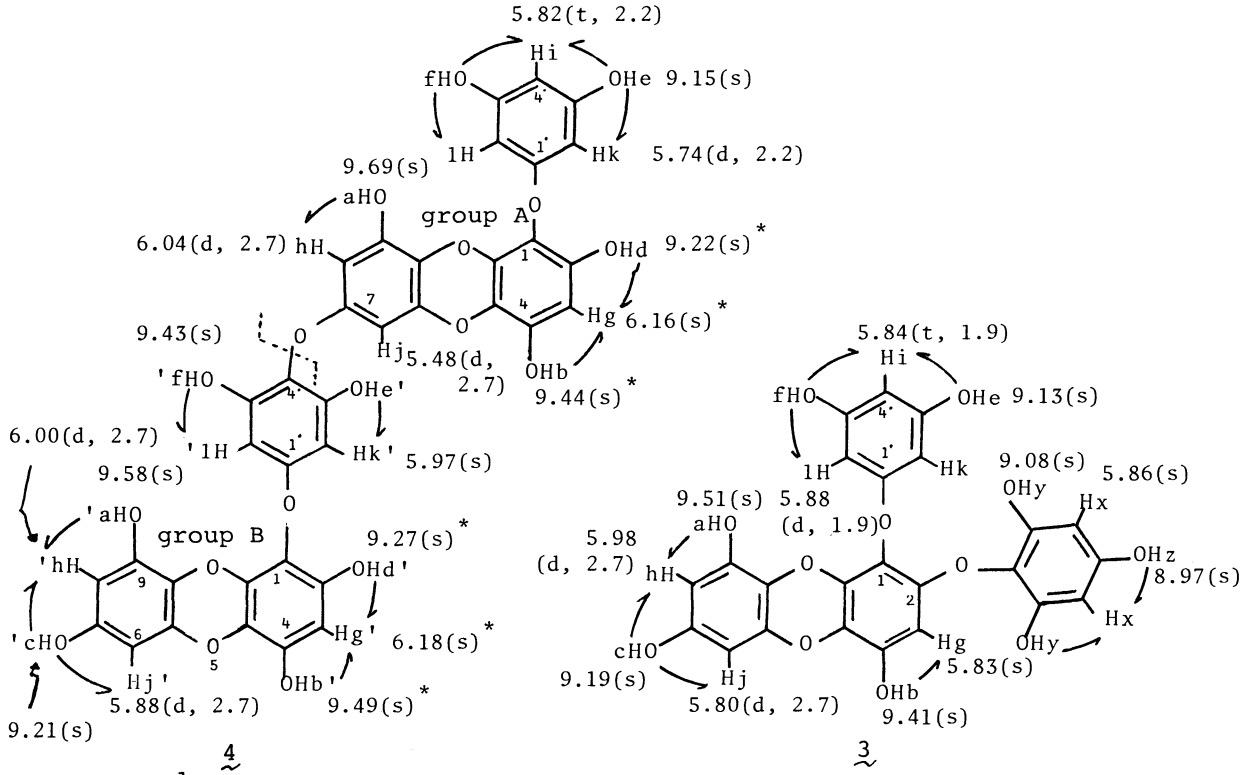
Dieckol (4), mp >300 °C, has the following data: λ_{max}(MeOH) 235(ε 34000) and 292(ε 3500) nm; ν_{max}(KBr) 3250(OH) and 1605 cm^{-1}; FDMS m/z 742(M^+, $C_{36}H_{22}O_{18}$). The mass spectrum of 4 showed typical fragment peaks at m/z 264 and 232 derived from basic dibenzodioxin unit of eckol, disclosing that 4 is a dimer of 1. Analysis of

the NOE experiments (Fig. 3) revealed that the protons of 4 could be grouped in pairs corresponding to the protons of 1 except for OHc and Hi for each of which only one proton was absent. This immediately indicated that 4 arose from dimerization of 1 via a C-7 - C-4' ether bond. This conclusion was supported by the following facts: (1) the presence of an oxygen-bearing aromatic carbon atom (δ 124.6) assignable to C-4' in group B of 4, (2) the observation in the ^{1}H NMR spectrum of large downfield shifts for He', Hf', Hl', and Hk' relative to the corresponding values for 1, (3) the effect of substitution upon the ^{13}C resonances of C-7 in



1 2 3

Fig. 2. ^{13}C NMR data (δ/ppm) of 1, 2, and 3 in DMSO-d_6.



4 3

Fig. 3. ^{1}H NMR data of 3 and 4 in DMSO-d_6 and the observed negative NOEs are indicated by arrows. * Assignment may be interchangeable.

group A, C-1' and C-3' in group B (see Table 1). Thus, the structure of dieckol was established as 4. To our knowledge, the eckols described herein belong to a new class of phlorotannins with a dibenzo-p-dioxin skeleton and also are the first example of them isolated without any prior derivatization such as methylation or acetylation. It should be emphasized that the measurement of the negative NOEs is a valuable tool for the assignment of the protons including phenolic OH protons of polyhydroxylated phenols like the eckols. Inhibitory activities (IC_{50}) on α_2-macroglobulin (α-M) and α_2-plasmin inhibitor (α-PI) are as follows: 2.5 μg/ml(α-M) and 1.6 μg/ml (α-PI) for 1, 5.1 μg/ml(α-M) and 5.1 μg/ml(α-PI) for 3, 5.0 μg/ml(α-M) and 0.8 μg/ml(α-PI) for 4.[9]

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Table 1. ^{13}C NMR data of 4 (100.61 MHz, DMSO-d_6, TMS as int. standard)

Carbon	Group A	Group B
1	123.6 s	123.4 s
2	146.0 s	146.0 s
3	98.5 d	98.5 d
4	141.8 s	141.9 s
4a	122.6 s	122.7 s
5a	142.5 s	142.7 s
6	93.7 d	94.3 d
7	154.3 s	153.0 s
8	98.6 d	98.7 d
9	146.0 s	146.0 s
9a	124.3 s	122.8 s
10a	137.3 s	137.1 s
1'	160.3 s	155.9 s
2'	93.9 d	94.8 d
3'	158.7 s	151.0 s
4'	96.4 d	124.6 s

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4) H. Iwaki, Y. Nakayama, M. Takahashi, S. Uetsuki, M. Kido, and Y. Fukuyama, J. Antibiot., 37, 1091 (1984).
5) K. -W. Glombitza, M. Koch, and G. Eckhardt, Phytochemistry, 15, 1082 (1976). Triphloroethol A, B, and C were isolated after acetylation. From E. kurome, we obtained naturally occurring triphloroethol B (2): oil, ν_{max} 3350, 1620, and 1480 cm^{-1}; m/z 374(M^+), 250, 232, and 126.
6) S. Forsen and R. A. Hoffman, J. Chem. Phys., 40, 1189 (1964); J. R. Kalman and D. H. Williams, J. Am. Chem. Soc., 102, 906 (1980).
7) The structure of 1 has been confirmed by an X-ray analysis. Crystal data: $C_{18}H_{12}O_9 \cdot C_3H_6O$, triclinic, $P\bar{1}$, a=8.277(4), b=9.281(5), c=13.646(9)Å, α=108.41(5), β=80.91(5), γ=112.13(4)°, and Z=2. Final R value is 0.052.
8) The shift values of C-2, C-3, and C-4a in comparison of 1 and 3 are -1.7, 2.2, and -2.3, respectively.
9) The detailed biological properties for the eckols will be published elsewhere.

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