CONSTITUENTS OF *JUNCUS EFFUSUS*: THE X-RAY ANALYSIS OF EFFUSOL DIACETATE

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ABSTRACT.—Chemical examination of *Juncus effusus* Linné resulted in the isolation of two unusual 9,10-dihydrophenanthrenes, effusol (**2**) and the highly cytotoxic juncusol (**1**), as well as β-sitosterol and β-sitosterolglucoside. The structure of effusol was confirmed by chemical transformations and X-ray analysis of effusol diacetate.

The genus *Juncus* (Juncaceae) is distributed all over the world in coastal marsh lands as well as inland. As part of a program to investigate systematically the various species of the genus *Juncus* for new physiologically active 9,10-dihydrophenthrenes (1–3), we examined the fresh water marsh plant, *Juncus effusus* Linné. The finely ground aerial parts of *J. effusus* were extracted with 95% ethanol. The concentrated alcoholic extract was partitioned between chloroform and water. The chloroform extract was fractionated into five fractions (designated A–E) by counter current distribution (ccd). Column chromatography of fraction B on silica gel followed by crystallization from benzene afforded the known highly cytotoxic 9,10-dihydrophenanthrene, juncusol (**1**), mp. 175–176° (4–5). The identity of juncusol was confirmed by comparison with an authentic sample (mp, mmp, tlc, ^1H, and ^{13}C nmr spectra).

Fractions collected from column chromatography of fraction B on crystallization from benzene-acetone yielded effusol (**2**), $C_{17}H_{16}O_2$ (M^+252), mp. 210–211°. The infrared spectrum of effusol in nujol showed absorption at 3250 (hydroxyl), 1605 (aromatic) and 915 (vinyl) cm^{-1}. The 90 MHz ^1H nmr spectrum of effusol in $CDCl_3$ containing a few drops of acetone-d_6 exhibited a 3H sharp singlet at δ 2.23 for an aromatic methyl group and a 4H singlet at δ 2.62 typical of the methylene protons of the 9,10-dihydrophenanthrene ring system. The spectrum also showed ABX type of signals for a vinyl group consisting of three sets of "quartets" at δ 5.20 (1H, $J_{BX}=11$ Hz; $J_{AB}=2$ Hz), 5.63 (1H, $J_{AX}=17$ Hz, $J_{AB}=2$ Hz) and 6.95 (1H, $J_{AX}=17$ Hz, $J_{BX}=11$ Hz), two ortho-aromatic proton doublets at δ 6.73 ($J=8$ Hz) and 7.23 ($J=8$ Hz) and two meta-aromatic proton doublets at δ 6.76 ($J=1.5$ Hz) and 6.93 ($J=1.5$ Hz). The ^{13}C nmr spectrum of effusol exhibited eight singlets, five doublets, three triplets and one quartet corresponding to 17 carbons in the molecule. Table 1 shows the chemical shifts and assignments for effusol which are based on the direct analysis of the non-protonated carbons, partially and completely decoupled spectra, and by comparison with the spectra of juncusol and other related 9,10-dihydrophenanthrenes (6).

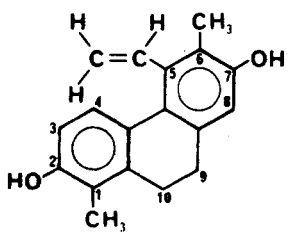
Treatment of effusol with acetic anhydride and pyridine afforded the diacetate **3**, mp 120–122°, revealing the presence of both the oxygen atoms as phenolic functions. Catalytic hydrogenation of effusol diacetate produced dihydroeffusol diacetate (**4**), mp 122–124°, in quantitative yield. The absence of the ABX signals and the appearance of a quartet at δ 2.84 and a triplet at δ 1.32 in the ^1H nmr spectrum of **4** confirmed the presence of the vinyl group in effusol. Upon deuteration with D_2O in the presence of potassium *t*-butoxide followed by acetylation, effusol afforded the tri- and di-deuterated (demonstrated by mass and ^1H and ^{13}C nmr spectroscopy) acetates **5** and **6**, respectively. The disappearance of the protons at C(3), C(6) and C(8), upon deuteration, confirmed the position of both hydroxyl groups in ring A and C of effusol. On the basis of the above spectral and chemical evidence, effusol has structure **2**.

While this work was in progress, Bhattacharyya reported (7) the isolation of a compound (effusol), mp. 177–178°, to which he assigned structure **2**. Except

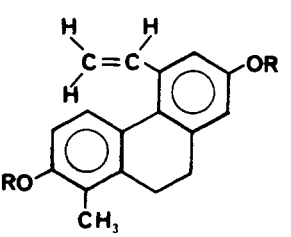
TABLE 1. ^{13}C nmr assignments for effusol and its acetate.

	Juncusol (1)	Effusol (2)	Effusol diacetate (3)
C(1)	120.2	121.1	126.7
C(2)	153.2	153.7	149.3
C(3)	111.1	113.1	119.8[b]
C(4)	128.6	127.5	119.2[b]
C(5)	139.9	140.6	140.9
C(6)	120.6	114.4	115.1
C(7)	153.4	155.2	148.4
C(8)	112.8	111.9	123.6
C(9)	30.3	30.5	25.3
C(10)	25.7	25.7	29.8
C(1)a	137.9	136.3	137.0
C(4)a	127.9	126.8	127.2[c]
C(5)a	127.7	126.2	127.6[c]
C(8)a	137.1	139.2	139.8[d]
CO	—	—	169.5
CH_3	—	—	20.9
C(1)-CH_3	11.8	11.7	12.6
C(6)-CH_3	13.2	—	—
CH	137.8	139.2	138.0
CH_2	119.9	113.4	118.7

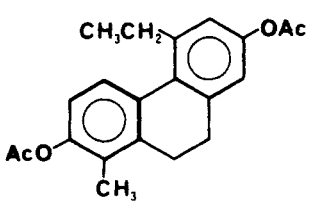
[b–c]Assignments may be reversed in the given vertical column.



1 Juncusol



2 R = H Effusol
3 R = Ac



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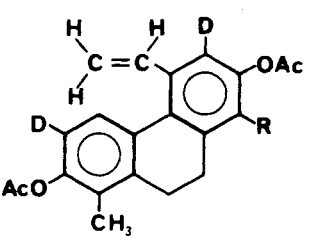

5 R = D
6 R = H

for the large discrepancy in melting point, all the data for Bhattacharyya's effusol appear to be identical with those of our compound.

To check the structure assigned to effusol, a single-crystal X-ray analysis was performed on the diacetate (3). A clear prismatic crystal of effusol acetate (3) with approximate dimensions of 0.3 x 0.3 x 0.3 mm was mounted on a glass fiber. The crystal was orthorhombic, with $a=7.998(2)$, $b=53.903(12)$, $c=8.267(1)$Å, and $d_{calcd.}=1.25$g cm^{-3} for eight molecules of $C_{21}H_{20}O_4$ in the unit

cell. The centrosymmetric space group Pbca was assigned on the basis of systematic extinctions in the data. Unique reflections with $\theta \leq 60°$ were measured on an Enraf-Nonius CAD–4 diffractometer using ω–2θ scans of width (.95+ .14 tan θ)°, a maximum scan time of 180 seconds per reflection, and CuKα radiation ($\lambda = 1.5418$Å). Intensities of three control reflections showed an approximate 10% decline during data collection, and scaling of the data set reflected this change. Of the 2633 reflections measured, 1453 were judged observed ($I \geq 2\sigma$ (I)) after application of Lorentz and polarization corrections.

The structure was solved by direct methods (8). Hydrogens were identified on difference maps after anisotropic refinement of the nonhydrogens and were isotropically refined (9). The quantity minimized during refinement was $w(\Delta F)^2$, where $w = (2.9 + |F| + 0.02F^2)^{-1}$. At convergence $R = 0.043$ and $R_w = 0.061$, and a final difference map was essentially featureless, showing no peaks greater than 0.17eÅ^{-3}.

The molecule is not planar (figure 1). Rather, the central ring has a twist-boat conformation considerably flattened at one end, and least-squares planes

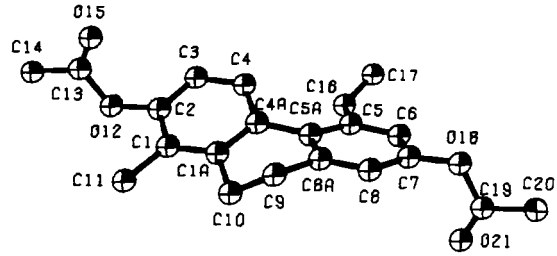

FIGURE 1. ORTP Drawing of Effusol Diacetate (3).

through the two aromatic rings intersect at an angle of 28°. Dihedral angles of the central ring are: C(4A)–C(1A)–C(10)–C(9) = 33.1(4)°; C(1A)–C(10)–C(9)–C(8A) = −54.6(3)°; C(10)–C(9)-C(8A)–C(5A) = 36.9(4)°; C(9)–C(8A)–C(5A)–C(4A) = 4.2(4)°; C(8A)–C(5A)–C(4A)–C(1A) = −27.8(4)°; and C(5A)–C(4A)–C(1A)–C(10) = 7.8(4)°. There are no unusual bond distances or angles and no short intermolecular contacts in the crystal structure. The crystal structure confirmed the structure assigned on the basis of chemical transformations and spectral data.

Biogenetically, occurrence of juncusol and effusol (norjuncusol) in the same plant is of interest. So far no biosynthetic work related to dihydrophenanthrene or phenanthrene natural products has been reported.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are corrected and were taken on a Thomas Kofler hot stage equipped with a microscope and polarizer. Infrared spectra were recorded on a Perkin-Elmer model 237B spectrophotometer. Proton nmr measurements were made in $CDCl_3$ solutions, unless otherwise mentioned, on a Varian EM–390 spectrometer with Me_4Si as an internal standard, and all the signals are reported as δ values. The following abbreviations are used to express the multiplicity of the signals: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet. The ^{13}C spectra were determined in $CDCl_3$ solution (which also provided the lock signal) on an FX–60 JEOL spectrometer, and the chemical shifts are reported in parts per million downfield from Me_4Si. Thin-layer chromatography (tlc) of compounds was accomplished on Merck silica gel 60 H, and the compounds were visualized in uv light and by spraying with conc. sulfuric acid. Column chromatography was conducted on Merck silica gel 60 F–254 (70–230 mesh). Preparative thick layer chromatography (ptlc) was carried out on 20 x 40 cm plates coated with a 2.5 mm layer of EM silica gel GF–254 and compounds were visualized in uv light. The solvent system used for tlc and plc was chloroform-methanol (90–95:10–5), unless otherwise mentioned.

EXTRACTION OF JUNCUS EFFUSUS.—The plant material used for this study was collected on highway 341, 10 miles north of Brunswick, Georgia, on May 20, 1979, while the plants were in the flowering stage. The identity of the plant was authenticated by the Department of Botany, University of Georgia Herbarium.

The dried plant material (11 kg) was milled and extracted four times with 95% ethanol (25 gallons) at room temperature. The ethanol extract was concentrated *in vacuo* to give 765 g of residue. The crude ethanolic extract (740 g) was partitioned between chloroform (5 x 2 liters) and water (1.5 liter). The total chloroform extract, when evaporated *in vacuo*, yielded 99 g of chloroform extract.

The chloroform extract was subjected to an 11-tube counter current distribution (ccd) using an upper phase of methanol-water (4:1, 1200 ml) and a lower phase of chloroform-carbontetrachloride (3:7, 1200 ml). Tube contents, when monitored by tlc and combined, as appropriate, yielded the following five fractions. Tubes 1 to 3—fraction A (35.61 g), tubes 4 to 6—fraction B (10.07 g), tubes 7 to 9—fraction C (13.17 g), tube 10—fraction D (16.38 g), and tube 11—fraction E (24.21 g).

COLUMN CHROMATOGRAPHY OF FRACTION B.—Fraction B (10 g) was dissolved in methylene chloride and placed on a column containing 300 g of silica gel and eluted successively with methylene chloride, 1% methanol/methylene chloride, 3% methanol/methylene chloride, 5% methanol/methylene chloride, 10% methanol/methylene chloride and finally with methanol (200 ml each fraction). The following fractions were obtained.

Fractions	Eluent
1–6	Methylene chloride
7–11	Methylene chloride
12–15	Methylene chloride
16–37	Methylene chloride
38–56	2% Methanol/Methylene chloride
57–72	5% Methanol/Methylene chloride
73–82	10% Methanol/Methylene chloride
83–86	100% Methanol

FRACTIONS 12–15: JUNCUSOL (**1**).—Crystallization of combined fractions 12–15 from acetone gave 40 mg of juncusol (**1**), with mp 175–176° (lit (4) mp 176°). The proton and ^{13}C nmr spectra were consistent with the published spectra (6). Finally, its identity was confirmed by comparison (mmp and tlc) with an authentic sample.

FRACTIONS 16–37: EFFUSOL (**2**).—The combined fractions 16 to 37 on further purification by filtration through a small amount of silica and crystallization from acetone-benzene afforded 200 mg of effusol (**2**), mp 210–211°, as a major compound of this fraction. The isolate exhibited the following spectral data, ir, λ max (nujol) 3250, 1605 and 915 cm^{-1}; ^1H nmr ($CDCl_3$ and few drops of acetone) 2.33 (3H, s, C-CH_3), 2.62 (4H, s, Ar-CH_2-CH_2-Ar), ABX type of signals for a vinyl group at 5.20 (1H, J=11 Hz, J=2 Hz), 5.63 (1H, J=17 Hz, J=2 Hz), and 6.95 (1H, J=17 Hz, J=11 Hz), and aromatic protons signals at 6.73 (1H, d, J=8 Hz), 7.23 (1H, d, J=8 Hz), 6.76 (1H, d, J=1.5 Hz), and 6.93 (1H, d, J=1.5 Hz); ^{13}C nmr ($CDCl_3$ and few drops of acetone) see table 1; *m/e* M^+ 252(95), 237(100), 219(70), 205(57), 181(44), 165(51), 152(31) and 108(28).

ACETYLATION OF EFFUSOL: EFFUSOL DIACETATE (**3**).—Effusol (**2**, 80 mg) was treated overnight with acetic anhydride-pyridine (1:1, 1 ml). Following the removal of excess reagents, the residue was purified by chromatography over 10 g of silica gel to afford an analytical sample of effusol diacetate, mp 120–122° (60 mg), after crystallization from methylene chloride-hexane mixture. The X-ray analysis was performed on a crystal of effusol diacetate (see figure 1). The diacetate exhibited the following spectral data, ir, 1750, 1603, 910, 880, and 840 cm^{-1}; ^1H nmr ($CDCl_3$) δ 2.18 (3H, s, Ar-CH_3), 2.30 (3H, s, -COCH_3), 2.32 (3H, s, -COCH_3), 2.80 (4H, s, Ar-CH_2-CH_2-Ar), 5.30 (1H, d), 5.44 (1H, d), 6.90 (1H, d), 7.10 (1H, d), 7.30 (1H, d), 7.70 (1H, d); ^{13}C nmr ($CDCl_3$) see table 1; M^+ (336).

DEUTERATION OF EFFUSOL.—A mixture of effusol (60 mg), potassium *tert*-butoxide and D_2O was heated in a sealed nitrogen-filled tube at 100° for three days. The solvent was evaporated, and the product was acetylated (acetic anhydride-pyridine at room temperature for 24 hours). Following the removal of excess reagents, the residue, when crystallized from methylene chloride-hexane mixture, yielded two different types of crystals. The nmr spectrum of the major product **6** (40 mg) indicated that two aromatic protons had exchanged with deuterium; M^+ 338(11) with fragmentation at *m/e* 297(58), 254(89), 239(100), 221(19), 210(16), 191(12), 167(11) and 154(9). The nmr of the minor product **5** revealed that three aromatic protons had exchanged with deuterium; M^+ 339(5), with fragmentation at *m/e* 297(37), 254(69), 239(100), 221(12), 210(15), 191(12), 167(16), and 154(13).

HYDROGENATION OF EFFUSOL DIACETATE: DIHYDROEFFUSOL DIACETATE (**4**).—Effusol acetate (20 mg) was hydrogenated in methanol solution (25 ml) with 5% Pd/C (30 mg) in the presence of hydrogen for four hours at room temperature. The solution was then filtered. The residue after evaporation was dissolved in methylene chloride and filtered again. The mother liquor, on standing, yielded a crystalline residue, mp. 122–124° in quantitative yield. The product exhibited the following spectral properties, ^1H nmr ($CDCl_3$) δ 1.32 (3H, t, CH_3-CH_2-Ar), 2.17 (3H, s, CH_3-Ar), 2.28 (3H, s, CH_3-CO), 2.30 (3H, s, CH_3-CO), 2.70 (4H, s, CH_2-CH_2Ar), 2.84

(2H, q, CH_3-CH_2-Ar), and aromatic protons at δ 6.80 (1H, *d*), 6.90 (1H, *d*), 7.22 (1H, d) and 7.41 (1H, d); M^+ 338.

ISOLATION AND IDENTIFICATION OF β-SITOSTEROL.—Column chromatography of Fraction E obtained from ccd, resulted in the isolation of β-sitosterol. Crystallization of several fractions from methanol afforded a white crystalline residue (100 mg) of mp 134–135°. The identity of β-sitosterol was confirmed by comparison (mmp and tlc) with an authentic sample.

ISOLATION AND IDENTIFICATION OF β-SITOSTEROLGLUCOSIDE.—Column chromatographic separation of Fraction E obtained from ccd, gave β-sitosterolglucoside as a white powder of mp 193–194°. The identity of this compound was confirmed by comparison (mmp and tlc) with an authentic sample.

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