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STRUCTURE AND STEREOCHEMISTRY OF EUPAFORMOSANIN, A NEW ANTILEUKEMIC AND ANTISARCOMA GERMACRANOLIDE FROM *EUPATORIUM FORMOSANUM**

KUO-HSIUNG LEE,†‡ TAKEATSU KIMURA,‡ MITSUMASA HARUNA,‡ ANDREW T. MCPHAIL,†§ KAY D. ONAN§ and HUAN-CHANG HUANG‖

‡Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514; §Paul M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27706; ‖School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China

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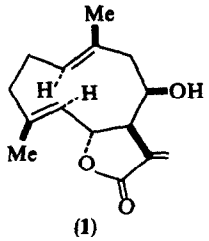
Key Word Index—*Eupatorium formosanum*; Compositae; eupaformosanin; antitumor sesquiterpene lactone; stereochemistry.

We reported recently [1,2] on the isolation and structure determination of two new cytotoxic agents, eupatolide (**1**) and eupaformonin (**2**), from *Eupatorium formosanum* Hay gathered in early spring 1973 in Wootai, Pingtong, Taiwan. Further investigations on those ethanolic fractions of this same plant which showed *in vivo* activity against Walker 256 carcinosarcoma as well as P-388 lymphocytic leukemia [3] have now led to the isolation and characterization of a new potent antileukemic antitumor principle, eupaformosanin (**3**).

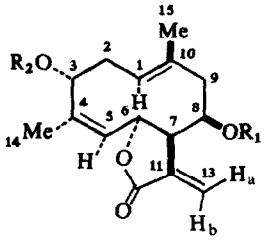
Eupaformosanin [(**3**), colourless needles, mp 91° (from MeOH–H_2O 1:3 or benzene), *m/e* 420.1781(M^+), $[\alpha]_D^{21}$ − 99.50° (*c* = 2,193, $CHCl_3$)] has molecular formula $C_{22}H_{28}O_8$. The presence of an α-methylene-γ-lactone moiety was indicated by the appearance of IR bands ($CHCl_3$) at 1755 and 1658 $cm.^{-1}$, and was substantiated by the presence in the ^1H- and ^{13}C-NMR spectra of a characteristic pair of low field doublets at δ 5.81 (1H, *J* = 2.0 Hz, H_a-13) and 6.38 (1H, *J* = 2.0 Hz, H_b-13), in addition to a triplet at δ_C 125.2 (C-13). The identity of the H-7 signal as a broad singlet at δ 3.01 was established by spin decoupling at the frequencies of H_a and H_b. Irradiation at the frequency of H-7 collapsed H_a and H_b into singlets and also sharpened the broad singlet at δ 5.30 (3H, H-6, H-5, and H-1) as well as the multiplet at δ 5.21 (1H, H-8). Further irradiation at the frequency of H-8 (δ 5.21) sharpened the broad singlet at δ 3.01 (H-7) and collapsed two well-separated doublets of doublets at δ 2.39 (1H, *J* = 3, 14 Hz, partially overlapped) and 2.73 (1H, J = 3, 14 Hz, partially overlapped) into two doublets (J = 14 Hz), thereby demonstrating that H-8 was adjacent to two protons at C-9 which in turn was adjacent to a fully substituted C-10. The foregoing observations were further confirmed by ^1H noise-decoupled ^{13}C FT NMR of (**3**) which showed C-6, C-7, C-8, and C-9 at 74.6 (*d*), 48.8 (*d*), 70.8 (*d*), and 43.3 (*t*), respectively. Irradiation of the broad singlet at δ 5.30 (3H, H-6, H-5, and H-1) not only caused a sharpening of the one-proton singlet at δ 3.01 (H-7), but also resulted in a sharpening of two three-proton broad singlets at δ 1.79 and 1.90, which could thus be assigned as two vinyl methyls at C-4 and C-10 [7], respectively.

* Antitumor Agents. 26. † Address correspondence to any of these authors.

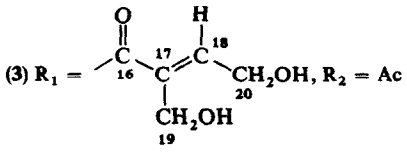
The IR spectrum of (3) exhibited two strong carbonyl bands at 1730 ~ 1720 cm^{-1}; one of these was due to an acetyl group as was also indicated by a ^{1}H-NMR signal at δ 2.12 (3H, s), the other was from an ester moiety. The presence of two primary hydroxyl groups in the ester side chain of (3) was revealed by the appearance of a strong IR band at 3420 cm^{-1}, a two-proton singlet at δ 4.36 (H-19) and a two-proton doublet at δ 4.43 (J = 6.0 Hz, H-20) in the ^{1}H-NMR spectrum. These primary hydroxyl groups were also seen in the ^{1}H noise-decoupled ^{13}C-NMR spectrum as two triplets at δ_C 59.1 and 56.9. The one-proton signal at δ 6.95 (t, J = 6.0 Hz) was assigned to the hydrogen at C-18 since irradiation at H-20 (δ 4.43) caused the signal for H-18 to collapse to a singlet. The above evidence led to the assignment of a 2-hydroxymethyl-4-hydroxy-2-butenoate structure for the ester group as shown in (3). The remaining low-field signal at δ 5.61 (1H, *dd*, J = 5.5 and 12.0 Hz) was ascribed to a proton attached to a carbon which bore an acetoxy group, i.e. H-3, by off-resonance proton decoupling in the ^{13}C-NMR spectrum which showed a doublet at δ_C 79.7. The triplet at δ_C 30.7 was thus assigned to the carbon at the 2-position. Considerations from the biogenetic implications observed in the co-occurrence of eupaformosanin (3) and eupaformonin (2), coupled with the evidence presented above, led to the formulation of structure (3) for the former.

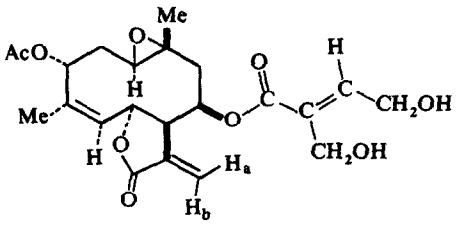


(1)



(2) R_1 = H, R_2 = Ac





(4)

Epoxidation of (3) with *m*-chloroperbenzoic acid afforded eupaformosanin epoxide [(4), mp 158° (from $CHCl_3$–Et_2O), $C_{22}H_{26}O_8$, *m/e* 418.1628 $(M-H_2O)^+$, 376 (M-60), 368, 358, 305 (M-131) and 244 (M-192)]. Extensive double resonance experiments led to the following assignment of protons consistent with structure (3): δ 5.85 (1H, J = 2.0 Hz, H_a-13), 6.41 (1H, J = 2.0 Hz, H_b-13), 5.35 (1H, *d*, J = 11.5 Hz, H-5), 5.69 (1H, *dd*, J = 2.0 and 11.5 Hz, H-6), 2.94 (1H, *br. s*, H-7), 5.25 (1H, *br. s*, H-8), 2.52–2.76 (2H, *m*, H-9), 2.85 (1H, *d*, J = 12.0 Hz, H-1), 2.20–2.40 (2H, *q*, J = 5.0 and 12.0 Hz, H-2), 5.89 (1H, *dd*, J = 5.5 and 12.0 Hz, H-3), 1.86 (3H, *d*, J = 1.0 Hz, H-14), 1.58 (3H, *br. s*, H-15), 2.11 (3H, *s*, OCOMe), 6.99 (1H, *t*, J = 6.0 Hz, H-18), 4.36 (2H, *s*, H-19), and 4.43 (2H, *d*, J = 6.0 Hz, H-20).

Unequivocal proof of the structure and stereochemistry of (3) was provided by single-crystal X-ray analysis of the 1,10-epoxide (4) which crystallizes in the monoclinic system, space group $P2_1$, with a = 11.559(7), b = 6.288(5), c = 15.057(8) Å, β = 97.04(5)°, Z = 2. The structure was solved by direct methods by use of Multan [4]. Full-matrix least-squares refinement of positional and anisotropic thermal parameters for the carbon and oxygen atoms (hydrogen atoms have been included at their calculated positions) has resulted in an R value of 0.087 over 1258 statistically significant [$I > 2.0\sigma(I)$] reflections measured on an Enraf-Nonius CAD 3 diffractometer (Ni-filtered Cu-K_α radiation, λ = 1.5418 Å; θ-2θ scans]. Bond lengths and valency angles agree with expected values. The ten-membered ring, characterized by endocyclic torsion angles $\omega_{1,2}$ − 85.5, $\omega_{2,3}$ − 53.0, $\omega_{3,4}$ 90.6, $\omega_{4,5}$ − 4.8, $\omega_{5,6}$ − 124.7, $\omega_{6,7}$ 138.1, $\omega_{7,8}$ − 70.4, $\omega_{8,9}$ 52.2, $\omega_{9,10}$ − 84.4, $\omega_{10,1}$ 158.6°, has a chair-chair-boat conformation similar to those found in eupaformonin (2) [2] where the corresponding angles are − 80, − 58, 92, 4, − 124, 135, − 80, 61, − 96, 168° and dihydroheliangine [5] for which the values are − 88, − 46, 80, − 4, − 122, 149, − 80, 58, − 84, nd 163°.

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VALERANON-GEHALT IN DEN UNTERIRDISCHEN TEILEN VON *NARDOSTACHYS JATAMANSI* UND *VALERIANA OFFICINALIS*

HEINZ HÖRSTER*, GERHARD RÜCKER† und JOACHIM TAUTGES†
*Institut für Pharmazeutische Biologie und Phytochemie;
†Institut für Pharmazeutische Chemie der Westfälischen Wilhelms-Universität, Münster, BRD

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Key Word Index—*Nardostachys jatamansi*; *Valeriana officinalis*; valeranone.

Aus den unterirdischen Teilen von *Valeriana officinalis*, *V. wallichii* DC., *Nardostachys jatamansi* DC. und *N. chinensis* Batalin sind Verbindungen mit unterschiedlichen pharmakologischen Wirkungsprofilen isoliert worden [1–4] welche die Wirkung der Gesamtextrakte nicht vollständig widerspiegeln. Es gibt Hinweise, daß auch dem Sesquiterpen-Keton Valeranon (**1**) in dieser Hinsicht Bedeutung zukommt [3, 5], für das tranquillierende und antiulzerogene Eigenschaften beobachtet wurden [3, 6]. Es war deshalb interessant, den Gehalt von **1** in *N. jatamansi* und *V. officinalis* zu ermitteln.

Die Verbindung wurde gc-rein aus einer Probe *N. jatamansi* isoliert [5, 6], die einen Gehalt von *ca* 25% (bezogen auf den Ätherextrakt; *ca* 1.1% in der lufttrockenen Droge) aufwies. Zur Klärung, ob dieser hohe Gehalt typisch für *N. jatamansi* ist und welche Ergebnisse aus einem statistischen Vergleich mit dem Gehalt in *V. officinalis* resultieren, wurde **1** in je 15 Proben *N. jatamansi* und *V. officinalis* nach der Methode des inneren Standards bestimmt. Die Werte zeigten eine Abweichung von der Normalverteilung, so daß für die statistische Bearbeitung der verteilungsfreie Homogenitätstest nach Kolmogoroff [7] und Smirnoff [8] (Zit. in Sachs [9], verwendet wurde (Tab. 1).

Da mit D = 6/15 die kritische Prüfgröße $D_{max\,0,05}$ = 8/15 nicht erreicht wurde, kann nicht verallgemeinert werden, daß bezüglich **1** die Proben von *N. jatamansi* sich bedeutsam von Proben aus *V. officinalis* unterscheiden. Die Verteilungskurve der Gehalte von **1** in *N. jatamansi* zeigt neben der auffallend großen Variabilität der Werte (0,3–33% bezogen auf den Ätherextrakt: 0,01–1,2% in der lufttrockenen Droge), zwei deutliche Maxima, die anzeigen, daß die Proben von mindestens zwei sehr verschiedenen Kollektiven stammen. Ob diese Variabilität durch äußere Faktoren (Ernteprozedur, Lagerung der Droge usw.) oder durch physiologische bzw. genetische Faktoren verursacht wird, kann z. Zt. nicht geklärt werden. Die Gehalte an **1** in *V. officinalis* sind einheitlich sehr niedrig und übersteigen in keinem Fall den Wert von 1,5% (bezogen auf den Äther-Extrakt; *ca* 0,004% in der lufttrockenen Droge).

EXPERIMENTELLES

50 g grob gepulverte unterirdische Teile wurden 24 hr in einer Soxleth-Apparatur mit Äther extrahiert und das Lösungsmittel

Tabelle 1. Durchführung und Ergebnis des Homogenitätstests nach Kolmogoroff und Smirnoff [7–9]

$$D = \max\left(\frac{F_1}{n_1} - \frac{F_2}{n_2}\right)$$

F_i = Summenhäufigkeit d. Merkmals
n_i = Anzahl der Stichproben der verschiedenen Kollektive

Klasseneinteilung: Valeranongehalt im Äther-Extrakt	3,5	7,0	10,5	14,0	17,5	21,0	24,5	28,0	31,5	35,0	%
V. officinalis n	15	0	0	0	0	0	0	0	0	0	
N. jatamansi n	9	1	0	0	0	0	2	1	1	1	
F_1/n_1 V off	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
F_i/n_i N. jat.	9/15	10/15	10/15	10/15	10/15	10/15	12/15	13/15	14/15	15/15	
$\frac{F_1}{n_1}$	6/15	5/15	5/15	5/15	5/15	5/15	3/15	2/15	1/15	0/15	

Prüfgröße $D_{max} = \frac{6}{15} < D_{max}\,0.05 = \frac{8}{15}$