(Kavalier Votice, ČSSR; 25 × 250 mm) with a gradient elution (*n*-hexane–ethyl acetate, 10 ml/min. Mps were measured on a Kofler block, NMR in $CDCl_3$, IR spectrum in CCl_4, MS on AEI-902, CD and ORD were measured in dioxane.

Isolation of **1**. The $CHCl_3$ extract (39.16 g) of dried *N. scalaris* (4190 g), collected in June 1976 in Smědava (Iser Mountains), was chromatographed on Si gel; the fraction eluted with C_6H_6 + 1% Et_2O was chromatographed on a HPLC column. The fraction eluted by 4% EtOAc in *n*-hexane (39.8 mg) yielded **1**, which showed mp 213–215°, elemental composition $C_{31}H_{50}O_2$ (HRMS), MS: 454, 439, 422, 407, 307. IR cm^{-1}: 1702 (CO), 1612 and 1660 (C=C), 1364 and 1386 (gem diMe), 1426 ($\underline{CH}_2$—C=O); $[\alpha]_D^{25}$ +81° (dioxane); ORD: ϕ_{307} +3100, ϕ_{282} 0, ϕ_{266} −460, ϕ_{243} −180, ϕ_{225} −3890; CD: $\Delta\varepsilon_{292}$ +0.82, $\Delta\varepsilon_{250}$ 0, $\Delta\varepsilon_{210}$ −9.25. NMR: see [14].

Acknowledgements—Our thanks are due to Dr. J. Váňa, Faculty of Sciences, Charles University, Prague, for collection and identification of the plant material; Dr. S. Vašíčková for measurement and interpretation of the IR, ORD and CD spectra; and to Dr. L. Dolejš for measurement and interpretation of the MS.

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Phytochemistry, Vol. 20, No. 11, pp. 2592–2594, 1981.
Printed in Great Britain.

0031-9422/81/112592-03 $02.00/0

GENKWADAPHNIN, A POTENT ANTILEUKEMIC DITERPENE FROM *DAPHNE GENKWA**

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(*Revised received* 23 *April* 1981)

Key Word Index—*Daphne genkwa*; Thymelaeaceae; genkwadaphnin; antileukemic diterpene.

Abstract—*In vivo* P-388 assay-directed fractionation of an active extract of *Daphne genkwa* (Yuán Huā) has led to the isolation and characterization of a new antileukemic principle, genkwadaphnin.

INTRODUCTION

The flowers of *Daphne genkwa* Sieb. et Zucc. (Thymelaeaceae) are known as 'Yuán Huā' in Chinese folklore and as herbal remedies for human diuresis for centuries [1–3] as well as for cancer recently [4]. Previous chemical studies on this drug resulted in the isolation of genkwanin, apigenin, sitosterol and benzoic acid [5]. As a result of the continuing search among Chinese plants for new naturally occurring potential antitumor agents, a methanolic extract of Yuán Huā was found to show significant inhibitory activity *in vivo* against the P-388 lymphocytic leukemia in mice. We report herein the isolation and structural elucidation of the new principal antileukemic constituent, genkwadaphin (**1**) from this active extract.

RESULTS AND DISCUSSION

The *in vivo* P-388 assay-directed fractionation of the active extract of *D. genkwa* led to the isolation of **1** as an antileukemic component. The spectral data described in

*Part 40 in the series "Antitumor Agents". For Part 39 see Lee, K. H., Tagahara, K., Suzuki, H., Wu, R. Y., Haruna, M., Hall, I. H., Huang, H. C., Ito, K., Iida, T. and Lai, J. S. *J. Nat. Prod.* (in press).

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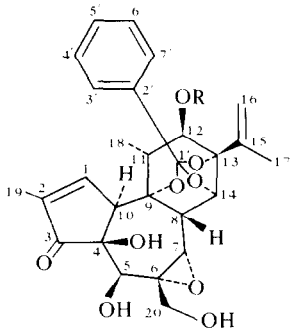

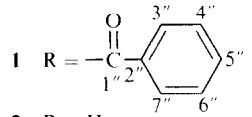

1 R = 2″-benzoyl

2 R = H

the Experimental suggested that **1** was structurally closely related to the daphnane-type diterpenes, isolated from other *Daphne* [7–9] and *Gnidia* [10–13] species of the Thymelaeaceae as well as *Hura crepitans* [13–15], *Hippomane mancinella* [16], and *Excoecaria agallocha* [17] of the Euphorbiaceae [18]. A comparison of the aforementioned physicochemical data with those described for 12-*O*-benzoyl-12-hydroxydaphnetoxin [10], a derivative prepared by selective benzoylation of 12-hydroxydaphnetoxin (**2**) which was obtained as a hydrolysed product of the natural gnidicin, isolated from *Gnidia lamprantha* [10], indicated the identity of both compounds. Further confirmation of the structure of **1** as 12-*O*-benzoyl-12-hydroxydaphnetoxin was achieved by saponification of **1** with 0.1 N methanolic potassium hydroxide at room temperature to yield a benzoic acid methyl ester and a desbenzoyl compound (**2**) which was identified as 12-hydroxydaphnetoxin by comparative UV, IR, ^{1}H NMR and MS spectral analyses. Such criteria left no doubt that the structure of the antileukemic genkwadaphnin could be assigned to **1**. Genkwadaphnin demonstrated significant ($T/C \geq 120\%$) antileukemic activity at low dose in P-388 leukemia (e.g. $T/C = 175$, 149, 140, 131% at 0.8, 0.4, 0.2 and 0.1 mg/kg, respectively).* Studies on the structure–antileukemic activity relationships and the mechanism of action of genkwadaphnin related compounds are in progress.

EXPERIMENTAL

Mps were determined on a Thomas–Hoover melting point apparatus and were uncorr. Specific rotations were obtained on a Rudolph Autopol III automatic polarimeter (l = 0.5 dm). ^{1}H NMR spectra were determined with Me_4Si as an int. standard. ^{13}C NMR spectra were recorded at 25.20 MHz. All NMR spectra were obtained with the use of the Fourier transform technique. Mass spectra were determined on an AEI MS-902 instrument at 70 eV using a direct inlet system. SilicAR-CC7 Special (Mallinckrodt) and Sephadex LH20 (Pharmacia) were used for CC. Precoated Si gel GF (Analtech Uniplate, 1000 μm) was used for prep. TLC. Detection of components was made either by spraying with 1% cerium sulfate–10% H_2SO_4 followed by heating or by use of a UV lamp. High-performance liquid chromatography (HPLC) was performed on a Waters Associates Model ALC/GPC 244 Liquid Chromatograph using a Whatman Partisil M9 10/50 column and a mixture of *n*-hexane–isopropanol (5:1). The *in vivo* activity was assayed by Dr. I. H. Hall, Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina at Chapel Hill by a lit. method [6].

Isolation of genkwadaphnin (**1**). The air-dried flowers of *D. genkwa* were extrd with MeOH. Guided by the *in vivo* P-388 assay, the resulting active residue was dissolved in MeOH–H_2O (1:1) and then partitioned into *n*-hexane, Et_2O and $CHCl_3$ successively. CC of the active ethereal layer on Si gel and Sephadex in $CHCl_3$ afforded a fraction which concentrated the antileukemic activity. Subsequent purification of this active fraction by prep. TLC and HPLC led to the isolation of pure antileukemic genkwadaphnin as a colorless amorphous substance (**1**, 0.00001% yield): $[\alpha]_D^{23}$ +63.8° (*c* 0.92, $CHCl_3$); UV_{max} (EtOH) 230 nm (ε 19 600); IR ($CHCl_3$) 3470, 1710, 1700, 1630, 1270 and 1105 cm^{-1}; MS *m/z* 602.2154 (M^+, Calc. for $C_{34}H_{34}O_{10}$: 602.2152); ^{1}H NMR ($CDCl_3$) δ 1.43 (3 H, *d*, *J* = 7 Hz, 18-H), 1.72 (3 H, *s*, 19-H), 1.90 (3 H, *s*, 17-H), 2.63 (1 H, *q*, *J* = 7 Hz, 11-H), 3.62 (1 H, *s*, 7-H), 3.80 (4 H, 8-H, 10-H, 20-H_2), 4.16 (1 H, *s*, 5-H), 5.05 (3 H, 14-H, 16-H_2), 5.28 (1 H, *s*, 12-H), 7.50 (1 H, *s*, 1-H) and 7.20–8.05, *m*, aromatic-H); ^{13}C NMR ($CDCl_3$) δ 9.9 (17-C), 18.4 (18-C), 18.8 (19-C), 35.7 (8-C), 44.1 (11-C), 47.4 (10-C), 61.0 (6-C), 64.0 (7-C), 64.8 (20-C), 71.3 (5-C), 72.4 (9-C), 78.5 (4-C), 78.9 (12-C), 80.7 (14-C), 84.3 (13-C), 113.8 (16-C), 117.9 (1′-C), 126.1 (3′-C, 7′-C), 128.1 (4′-C, 5′-C, 6′-C), 128.6 (4″-C, 6″-C), 129.5 (2′-C), 129.7 (3″-C, 7″-C), 133.3 (5″-C), 135.2 (2″-C), 136.9 (2-C), 142.9 (15-C), 160.1 (1-C), 165.5 (1″-C) and 209.2 (3-C).

Acknowledgements—This investigation was supported by grants from the National Cancer Institute (CA 17625) and the American Cancer Society (CH 19) to K.H.L.

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*We thank Dr. Matthew Suffness of the National Cancer Institute for kindly providing us these screening data (i.e. T/C = 149, 140 and 131% at 0.4, 0.2 and 0.1 mg/kg, respectively) for **1**.

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Phytochemistry, Vol. 20, No. 11, pp. 2594–2595, 1981.
Printed in Great Britain.

0031-9422/81/112594-02 $02.00/0

COMMENTS ON THE STRUCTURE AND SYNTHESIS OF JASMINOL, A TRITERPENE REPORTED FROM *JASMINUM AURICULATUM*

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(*Revised received* 20 *April* 1981)

Key Word Index—*Jasminum auriculatum*; Oleaceae; triterpenes; jasminol; olean-12-en-3β-ol.

Abstract—The assignment of the lup-20(29)en-28-ol structure for jasminol, and subsequent synthetic proof, are shown to be insecure.

In 1970, a report [1] appeared describing the structure of jasminol, a new triterpene from *Jasminum auriculatum* (Vahl), as lup-20(29)en-28-ol (**1**). The structural assignment was based on spectral data; subsequently, synthetic lup-20(29)en-28-ol was stated [2] to be identical with jasminol.

As regards the spectral data [1, 3] of the natural product, there are a number of features incompatible with the structure **1**. Among the major discrepancies, a ^{1}H NMR signal at *ca* δ 3.15 was assigned to the C-28 C$\underline{H}_2$OH protons, but these normally appear [4] in betulin (**2**) and related compounds as an AB quartet at *ca* δ 3.5 (ΔAB 14 Hz, J_{AB} = 11 Hz). Further, in the mass spectrum of jasminol, peaks at *m/z* 220 and 249 were ascribed to the well-known ring C cleavage modes of triterpenes. These peaks cannot, however, be readily reconciled with structure **1** since the ring C fragmentation peaks would be expected to appear at *m/z* 191, 204, 205 and 234 [5]. Finally, in 1970, two unambiguous syntheses of **1** were already on record [6, 7]. While no spectral data were given for the synthetic lup-20(29)en-28-ols, the reported mps and $[\alpha]_D$s deviate to a large degree from those given for jasminol (Table 1). Also, we have recently [8] developed a very short synthesis of **1**. The mp and $[\alpha]_D$ of our product are in accord with those given earlier [6, 7] for **1**; the ^{13}C NMR spectrum was identical to that published [9]; and the mass spectrum indeed showed peaks at *m/z* 191, 204, 205 and 234, while peaks at *m/z* 220 and 249 were absent. Finally, lup-20(29)en-28-ol has a much larger R_f (0.39) in TLC than that reported [10] for jasminol (0.22), whereas in our extract (see below) of *J. auriculatum* leaves, no component appeared on TLC within R_f 0.39 ± 20%. We thus conclude that the identity of jasminol with lup-20(29)en-28-ol is doubtful.

Table 1. Reported values of mp and $[\alpha]_D$ for various specimens of lup-20(29)en-28-ol (**1**)

Source	mp	$[\alpha]_D$	Ref.
Jasminol (natural product)	208–210°	+41.50	1
Ruzicka	140–141°	+16° ± 2°	6
Djerassi	140–142°	not given	7
Hase	144°	+16.8°	8
'Synthetic jasminol'	209°	+40°	2

Regarding the reported [2] synthesis of jasminol, it turns out that this synthesis is the same as had previously been reported by Djerassi [7] for the preparation of **1**, involving successive Huang-Minlon and $LiAlH_4$ reductions of methyl 3-oxobetulate. However, it is remarkable that while the spectra of the newly synthesized material clearly show that the product is indeed **1**, it was claimed to have a mp and $[\alpha]_D$ closely similar to those reported for jasminol, and unlike those given for **1** by Ruzicka [6] and Djerassi [7] and us [8] (Table 1). Finally, it should be mentioned that the synthetic paper [2] also contains the statement, "the identity was later confirmed by the usual procedure", without any supporting experimental details whatever. We conclude that lup-20(29)-28-ol (**1**) was certainly being synthesized