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ANTINEOPLASTIC AGENTS, 162.[1] *ZEPHYRANTHES CANDIDA*

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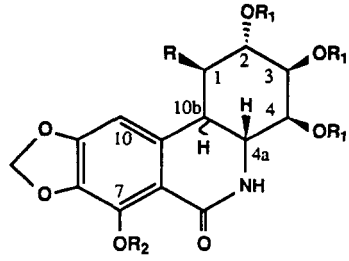
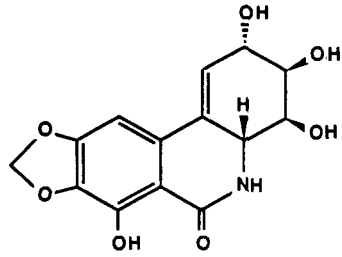
ABSTRACT.—The Chinese medicinal plant *Zephyranthes candida* was found to contain a cytostatic constituent. Separation of a *n*-BuOH extract directed by results of a bioassay employing the P-388 lymphocytic leukemia led to *trans*-dihydronarciclasine [**2**] as the principal cytostatic agent with ED_{50} 3.2×10^{-3} μg/ml.

Amaryllidaceous plants such as *Narcissus poeticus* were recorded in the Bible as well-established treatments for cancer (1), and others were in use by the Greek physicians of the fourth century BC (2). The first isolation, in 1877 (3), of a biologically active Amaryllidaceae constituent, the now well-known lycorine (4), was an early achievement of organic chemistry, and such studies have been intensifying (4–7). In 1984, we reported discovery and structural elucidation of a strongly antineoplastic phenanthridone designated pancratistatin [**1**] produced by plants of *Pancratium littorale* (2,8) and *Zephyranthes grandiflora* (5).

In 1964, extracts of the medicinal (5) *Zephyranthes candida* (Lindl.) Herb. (obtained in Hong Kong) had already proved active (KB cell line from a human epidermoid carcinoma of the nasopharynx) in the U.S. National Cancer Institute's exploratory research program, but we were unable to obtain a re-collection (People's Republic of China) until 1982. Earlier (1955) Boit and Ehmke (9) isolated four alkaloids from the Dutch *Z. candida* representing the pyrrolo[*de*]phenanthridine (lycorine), pretazettine (tazettine), and 5,10b-ethanophenanthridine (haemanthidine and nerinine) ring systems. The study was extended in 1964–65 (10,11) to isolation of dihydrolycorine and zephyranthine from a Japanese variety and in 1978 to a flavone glycoside (12). We now have found the principal cytostatic (murine P-388 lymphocytic leukemia, PS system) (12,13) constituent of *Z. candida* to be *trans*-dihydronarciclasine [**2**] previously (14) prepared by hydrogenation of narciclasine [**6**] and heretofore unknown as a biosynthetic product.



1 R=OH, R_1=R_2=H, 10b=α-H
2 R=R_1=R_2=H, 10b=α-H
3 R=H, R_1=R_2=Ac, 10b=α-H
4 R=R_2=H, R_1=Ac, 10b=α-H
5 R=R_1=R_2=H, 10b=β-H



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Ground bulbs of *Z. candida* were extracted with CH_2Cl_2-MeOH (1:1) at ambient temperature. After addition of H_2O, the aqueous phase was concentrated and extracted with *n*-BuOH. The PS-active (cell line) *n*-BuOH extract was concentrated and triturated with MeOH to provide a fraction that was separated (guided by PS bioassay) by successive

[1]For Part 161, see P.M. Blumberg and G.R. Pettit in BBA Reviews on Cancer, in preparation.

Sephadex LH-20 and Si gel cc steps. The resulting enriched active (ED_{50} 0.0034 μg/ml) fraction was devoid of acetate groups (ir), and the major component coeluted [tlc, CH_2Cl_2-MeOH-H_2O (90:10:0.1)] with an authentic synthetic specimen of *trans*-dihydronarciclasine. The fraction was acetylated and separated on a column of Si gel to yield *trans*-dihydronarciclasine peracetate [**3**] (PS ED_{50} 3.2×10^{-3} μg/ml) as the major component. The structure of the peracetate was established by detailed spectral analysis (2) and comparison with an authentic sample as well as with the product obtained by catalytic hydrogenation (Adam's catalyst in HOAc at 50 psi) of narciclasine, followed by acetylation. Hydrogenation afforded as the major product the expected *cis*-dihydronarciclasine accompanied by the trans isomer. Facile deacetylation of the phenolic acetoxy group was observed during chromatography and in MeOH solutions to give the 7-hydroxy-2,3,4-triacetoxy derivative **4**. *Trans*-dihydronarciclasine (prepared from the acetate) was found to strongly inhibit the PS leukemia with ED_{50} 0.0032 μg/ml, while the synthetic *cis*-dihydro analogue **5** led to PS ED_{50} 0.024 μg/ml.

Isolation of *trans*-dihydronarciclasine [**2**] as the major antineoplastic constituent of *Z. candida* has revealed another interesting and potentially useful Amaryllidaceae biosynthetic product. Further study of this very productive plant family for anticancer and other medically useful components will doubtless prove rewarding and is in progress.

EXPERIMENTAL

GENERAL METHODS.—Details of general procedures and chromatographic techniques were provided in our earlier summaries (2,5).

PLANT MATERIAL.—*Z. candida* PR #55337, NSCB657832 was re-collected in China in 1981 (received February 1982) as part of the NCI-USDA collaborative program directed by Drs. J.L. Hartwell and M. Suffness. A voucher specimen is maintained at the USDA, Beltsville, MD, and in the ASU-CRI.

Extraction.—Freshly ground bulbs (18 kg) were stored in MeOH-CH_2Cl_2 (1:1) (32 liters) for 10 days. Addition of H_2O (15% by volume) caused separation of the CH_2Cl_2 phase. MeOH and CH_2Cl_2 were added to the aqueous phase to increase the original total volume by 50 and 25%, respectively. The plant was extracted with this mixture (2:1:0.5 ratio of MeOH-H_2O to added MeOH and CH_2Cl_2) for a further 80 days. Addition of H_2O (25% by volume) allowed the CH_2Cl_2 phase to separate, which was combined with the first CH_2Cl_2 extract and concentrated to a 109-g residue (PS ED_{50} 3.5 μg/ml). The aqueous phase was concentrated and partitioned between H_2O (6 liters) and *n*-BuOH (4 × 6 liters). Concentration of the *n*-BuOH extract to a small volume and addition of MeOH (2 liters) gave an active MeOH-soluble fraction (149 g, PS ED_{50} 0.27 μg/ml). Upon further dilution with MeOH (600 ml) and CH_2Cl_2 (400 ml) the solution was filtered to yield 28 g of a solid (PS ED_{50} 1.6 μg/ml). The filtrate was chromatographed on a column of Sephadex LH-20 (2.5 kg) using MeOH-CH_2Cl_2 (3:2) as eluent.

ISOLATION OF *TRANS*-DIHYDRONARCICLASINE [**2**].—Elution (the preceding LH-20 column) between volumes 7215–16950 ml gave a 6.2-g fraction (PS ED_{50}<0.02 μg/ml). Trituration with Me_2CO (50 ml) provided a light orange solid (2.72 g, PS ED_{50} 0.016 μg/ml) and a soluble fraction (3.5 g, PS ED_{50} 0.0043 μg/ml). When the orange solid was triturated with MeOH-CH_2Cl_2 (1:1) (3 × 10 ml, 1 day), followed by MeOH (5 ml, 2 days), a soluble fraction (2.58 g) was obtained similar (by tlc) to the Me_2CO-soluble fraction. An aliquot of the Me_2CO-soluble fraction (1.76 g) and the latter soluble fraction (2.58 g) were combined and the mixture subjected to rapid chromatography on a column of Si gel (200 g). Gradient elution with CH_2Cl_2 (1 liter) and CH_2Cl_2-MeOH (99:1 to 95:5 to 9:1) (2 liters) gave a fraction (1.05 g) which was triturated with MeOH (5 ml, 1 day) to give a buff-colored solid [0.20 g, PS ED_{50} 0.0034 μg/ml, ir (KBr) 3350, 1660, 1460, 1340, 1280, 1225, 1060, 1025 cm^{-1}]. Half of the solid was acetylated [Ac_2O-pyridine (1:1) (6 ml), 24 h, room temperature], and the product (0.12 g) was chromatographed on a column of Si gel (Lobar B column). Development with CH_2Cl_2 (200 ml) and CH_2Cl_2-MeOH (99:1) (400 ml) followed by CH_2Cl_2-MeOH (49:1) (all affording between 675 and 725 ml) total eluent volume, *trans*-dihydronarciclasine-2,3,4-triacetate [**4**] (16 mg) which recrystallized from MeOH-CH_2Cl_2 as small colorless needles: mp 309–311° [lit. (13) mp 293°]; $[\alpha]^{32}D$ +81.94° (*c* = 0.72, $CHCl_3$); uv λ max MeOH (log ε) 231 (4.04), 239 (4.01), 280 (3.75), 310 (3.33) nm; ^1H nmr (400 MHz, $CDCl_3$) 1.914 (1H, ddd, *J* = 14.0, 12.5, 3.0

Hz, H-1β), 2.086 (6H, s, 2 × Ac), 2.137 (3H, s, Ac), 2.432 (1H, ddd, J = 14.0, 3.5, 3.2 Hz, H-1α), 3.134 (1H, ddd, J = 12.7, 12.5, 3.5 Hz, H-10b), 3.777 (1H, dd, J = 12.7, 11.8 Hz, H-4a), 5.175 (1H, dd, J = 11.8, 3.0 Hz, H-4), 5.189 (1H, m, H-3), 5.438 (1H, dd, J = 3.2, 3.0 Hz), 5.855 (1H, s, NH), 6.037, 6.049 (1H, each, d, J = 1.2 Hz, OCH_2O), 6.323 (1H, s, H-10), 9.704 (1H, s, ArOH). Acetylation [Ac_2O-pyridine (1:1)] led to *trans*-dihydronarciclasine peracetate [**3**] identified by tlc and ir spectra (in $CHCl_3$) with an authentic specimen.

Continued elution between volumes 725–760 ml gave a mixture (14 mg) of the above triacetate and *trans*-dihydronarciclasine peracetate and between volumes 760–810 ml *trans*-dihydronarciclasine peracetate (80 mg).

Recrystallization from $MeOH/CH_2Cl_2$ afforded a pure specimen of **3** as colorless needles: mp 181–182° [lit. (14) 188–189°]; $[\alpha]^{31}_D$ +123.9° (c = 1.13, $CHCl_3$) [lit. (14) $[\alpha]^{20}_D$ +128.5° (c = 0.82, $CHCl_3$)]; uv λ max MeOH (log ε) 231 (4.10), 239 (4.09), 280 (3.82), 310 (3.40) nm; ir (KBr) ν max 3600, 3500, 3330 (sh), 3310, 1760, 1730 (sh), 1670, 1634, 1505, 1487, 1460, 1371, 1345, 1298, 1255, 1235, 1172, 1080, 1051, 1031, 930 cm^{-1}; ^1H nmr (400 MHz, $CDCl_3$) 1.906 (1H, ddd, J = 14.0, 12.7, 3.0 Hz, H-1), 2.054, 2.071, 2.139 (3H each, Ac), 2.364 (3H, ArOAc), 2.428 (1H, ddd, J = 14.0, 3.5, 3.2 Hz, H-1α), 3.140 (1H, ddd, J = 12.7, 12.0, 3.5 Hz, H-10b), 3.762 (1H, dd, J = 12.0, 10.8 Hz, H-4a), 5.156 (1H, dd, J = 10.8, 3.0 Hz, H-4), 5.192 (1H, m, H-3), 5.416 (1H, dd, J = 3.2, 3.0 Hz, H-2), 5.810 (1H, s, NH), 6.065, 6.073 (1H each, d, J = 1.2 Hz, -O-CH_2-O), 6.642 (1H, s, H-10); ^{13}C nmr (22.63 MHz, $CDCl_3$) 170.34, 169.35, 169.14 (4C, 4 × OCOMe), 163.35 (C-6), 152.40 (C-9), 139.63 (C-7), 137.00 (C-10a), 134.33 (C-8), 116.04 (C-6a), 102.91 (OCH_2O), 102.00 (C-10), 71.62, 68.60, 67.43 (3 × *C*HOAc), 52.41 (C-4a), 35.61 (C-10b), 27.00 (C-1), 21.02, 20.86, 20.70 (4c, 4 × OCO*C*H_3) ppm; hreims m/z $[M]^+$ 477.1258 (3.09%) (calcd 477.1271 for $C_{22}H_{23}NO_{11}$), 435.1165 (100%) (calcd 435.1166 for $C_{20}H_{21}NO_{10}$).

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LITERATURE CITED

1. J.L. Hartwell, *Lloydia*, **30**, 379 (1967).
2. G.R. Pettit, V. Gaddamidi, D.L. Herald, S.B. Singh, G.M. Cragg, J. Schmidt, F.E. Boettner, M. Williams, and Y. Sagawa, *J. Nat. Prod.*, **49**, 995 (1986).
3. J.W. Cook and J.D. Loudon, in: "The Alkaloids." Ed. by R.H.F. Manske and H.L. Holmes, Academic Press, New York, 1952, Vol. II, Chapter 11, p. 331.
4. S. Ghosal, K.S. Saini, and S. Razdan, *Phytochemistry*, **24**, 2141 (1985).
5. G.R. Pettit, V. Gaddamidi, and G.M. Cragg, *J. Nat. Prod.*, **47**, 1018 (1984).
6. M. Kihara, T. Koike, Y. Imakura, K. Kida, T. Shingu, and S. Kobayashi, *Chem. Pharm. Bull.*, **35**, 1070 (1987).
7. H.-Y. Li, G.-E. Ma, Y. Xu, and S.-H. Hong, *Planta Med.*, 259 (1987).
8. G.R. Pettit, V. Gaddamidi, G.M. Cragg, D.L. Herald, and Y. Sagawa, *J. Chem. Soc., Chem. Commun.*, 1693 (1984).
9. H.G. Boit and H. Ehmke, *Chem. Ber.*, **88**, 1590 (1955).
10. S. Ozeki, *Yakugaku Zasshi*, **84**, 1194 (1964).
11. S. Ozeki, *Yakugaku Zasshi*, **85**, 200 (1965).
12. R.I. Green, N.H. Greenberg, M.M. MacDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep.*, **3**, 1 (1972).
13. M. Nakayama, T. Horie, M. Tsukayama, M. Masumura, and S. Hayashi, *Z. Naturforsch., C: Biosci.*, **33**, 587 (1978).
14. A. Mondon and K. Krohn, *Chem. Ber.*, **108**, 445 (1975).

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