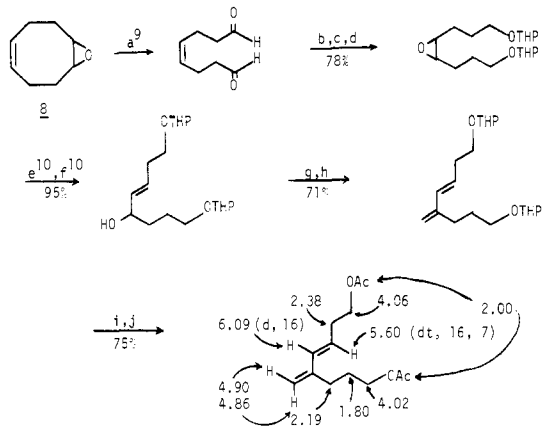
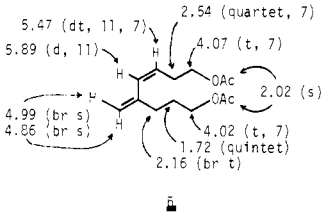
Scheme I[a]

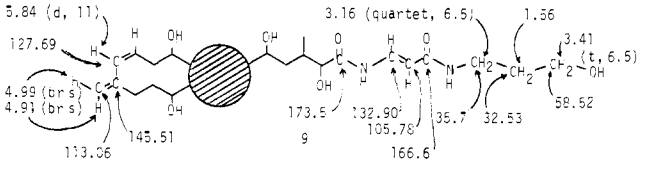


[a] a, $H_5IO_6$, 45 °C, 2 h; b, $NaBH_4$, EtOH, 0 °C; c, DHP, *p*-TsOH, room temperature, benzene; d, MCPBA, NaOAc, $CHCl_3$, room temperature, 12 h; e, $PhSe^-$, EtOH, Δ, 2 h; f, $H_2O_2$, room temperature, 6 h; g, DDO, $CH_2Cl_2$, room temperature, 12 h; h, $Ph_3P{=}CH_2$, THF, Δ. i, $H_3O^+$, $CH_3OH$. j, $Ac_2O$, pyridine.



ion, *m/e* 194 (10, M − $CH_2CO$ − $2H_2$), 180 (10, M − HOAc), 137 (5), 136 (6), 134 (7), 120 (68, M − 2HOAc), 108 (9), 107 (19), 105 (19), 94 (100, M − HOAc − $CH_2CHOAc$), 92 (27), 79 (16), 67 (3). The corresponding trans isomer **7** was readily synthesized from cyclooctadiene monoepoxide (**8**)[9] as outlined in Scheme I. Photoisomerization of **7** using naphthalene as the sensitizer gave a 1:1.3 mixture of **6** and **7** at the photostationary state which was reacted with maleic anhydride in benzene in a sealed tube (2 h, 90 °C, trace of hydroquinone). Separation of **6** and the Diels-Alder adduct of **7** was achieved by chromatography. The synthetic **6** was identical with **6** from the degraded toxin.

The palytoxins[2] therefore have partial structure **9** ($^1H$ and $^{13}C$ chemical shifts in $Me_2SO$-$d_6$).[11,12]



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**Supplementary Material Available:** The 90-MHz $^{13}C$ NMR spectrum of the palytoxin from a Tahitian *Palythoa* sp. in dimethyl sulfoxide-$d_6$ at 55 °C (4 pages). Ordering information is given on any current masthead page.

## References and Notes

(1) R. E. Moore and P. J. Scheuer, *Science*, **172**, 495–498 (1971).
(2) R. E. Moore, R. F. Dietrich, B. Hatton, T. Higa, and P. J. Scheuer, *J. Org. Chem.*, **40**, 540–542 (1975).
(3) Order Zoanthidea, subclass Zoantharia, class Anthozoa, phylum Coelenterata.
(4) The OH on C-1 is free in the palytoxins; i.e., it is not bound in a group such as a ketal. The proton and carbon-13 chemical shifts (in $Me_2SO$-$d_6$) of the methylenes in the 3-hydroxypropyl group of the palytoxins are identical with those of *N*-(3′-hydroxypropyl)-*trans*-3-acetamidoacrylamide; also, the C-1 carbon has a longer relaxation time ($T_1$) than any other proton-bearing carbon in the palytoxins.
(5) $^1H$ NMR studies of palytoxins at 360 MHz suggest that the second λ 233 chromophore is also a conjugated diene.
(6) $^1H$ NMR of major compound **3a** ($D_2O$): δ 7.81 (d, *J* = 14.5, H on C-7), 5.68 (d, *J* = 14.5 Hz, H on C-6), 5.24 (br t, *J* = 3 Hz, H on C-13), 3.68 (d, *J* = 10.5 Hz, H on C-10), 3.44 (t, *J* = 6 Hz, 2 H on C-1), 3.14 (t, *J* = 7 Hz, 2 H on C-3), 3.13 (s, OMe on C-13), 1.57 (quintet, 2 H on C-2), 0.92 (d, *J* = 7 Hz, Me on C-11). $^1H$ NMR of minor compound **3b** ($D_2O$): δ 7.49 (d, *J* = 14.5 Hz, H on C-7), 5.72 (d, *J* = 14.5 Hz, H on C-6), 3.13 (s, OMe), 3.12 (t, *J* = 7 Hz, 2 H on C-3), 0.72 (d, *J* = 7 Hz, Me).
(7) The two compounds in **2** are both labile in acid, but decompose at very different rates. In 0.07 N methanolic HCl the λ 263 absorption band disappears with a half-life of 100 h for **2a** and 19 h for **2b**.
(8) Mass spectrum (rel intensity) (20 eV) m/e 338 (1), 278 (8), 162 (2), 134 (100) with metastable ions at *m/e* 228.7 (338 → 278), 64.6 (278 → 134); high resolution mass spectrum *m/e* 338.145 (calcd for $C_{16}H_{22}N_2O_6$, 338.148), 278.124 (calcd for $C_{14}H_{18}N_2O_4$, 278.127), 134.063 (calcd for $C_6H_8NO$, 134.061).
(9) J. P. Nagarkatti and K. R. Ashley, *Tetrahedron Lett.*, 4599–4600 (1973).
(10) K. B. Sharpless and R. F. Lauer, *J. Am. Chem. Soc.*, **95**, 2697–2699 (1973).
(11) The $^{13}C$ NMR spectra were determined at 55 °C. Chemical shifts are relative to $Me_2SO$-$d_6$ (δ 39.63) as an internal standard. $^{13}C$–$^1H$ correlations are based on single-frequency off-resonance decoupling experiments at 90 MHz.
(12) The elemental composition of the palytoxin from Hawaiian *Palythoa toxica* has been roughly estimated to be $C_{145}H_{264}N_4O_{78}$.[1] Its 300-MHz or 360-MHz $^1H$ NMR spectrum is essentially identical with those of the palytoxins from Jamaican *P. mammilosa* and a Tahitian *Palythoa* sp. The differences are subtle. Minor differences can also be seen in the $^{13}C$ NMR spectra of these three toxins.[2] Integration of the $^1H$ NMR spectra suggests that there may be less than 264 protons in the palytoxins. In the proton noise-decoupled $^{13}C$ NMR spectra of the palytoxins at least 110 carbon peaks can be counted. Integration (under conditions to nullify nuclear Overhauser effects), however, indicates that there may be as many as 145 carbon atoms in the palytoxins.

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## Isolation and Characterization of the First Mitotic Cycle Hormone That Regulates Cell Proliferation

*Sir:*

Since the discovery of a natural substance that regulates cellular proliferation by Bullough and coinvestigators in the early 1960s, numerous investigators have attempted to clarify the chemical structures and physiological activities of various chalones.[1] The importance of these substances as tools in cancer research has become evident.

More recently, we (Evans and Van't Hof)[2] have characterized some physiological parameters of a G2 factor present in cotyledons of peas (*Pisum sativum*) that promotes cell arrest in G2[3] in both roots and shoots after seed germination. Many of the physiological responses of the G2 factor resemble those of chalones. The purpose of this communication is to describe the isolation and characterization of this G2 factor.

Seeds of garden peas (*Pisum sativum*) were surface sterilized and germinated on sterile vermiculite. The cotyledons (~3200) of the 3-day-old seedlings were aseptically excised and incubated in 8 L of sterile distilled water in 160 culture flasks for 2 days. The water extract was then filtered through a graded series of filters until it passed through a 0.30-mesh millipore filter. The extract was evaporated, chromatographed on Dowex 50W-X4,[4] dried, dissolved in 20% ethanol, and chromatographed on Sephadex LH-20.[4] The bioactive fractions which were assayed according to published methods[2] were pooled to give 10 g of residue. Two gel filtrations through Se-

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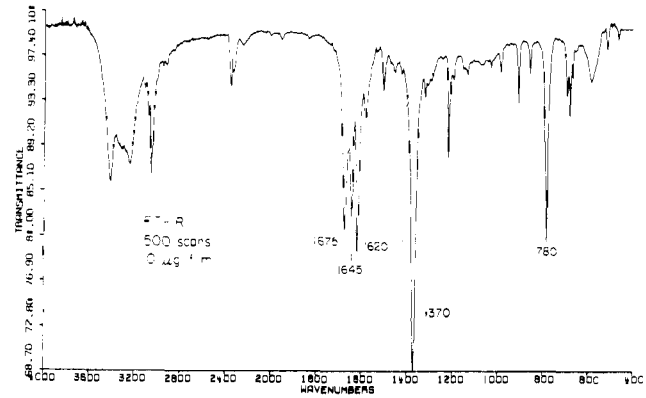


**Figure 1.** The FT IR of G2 factor, on KRS-5 plate.

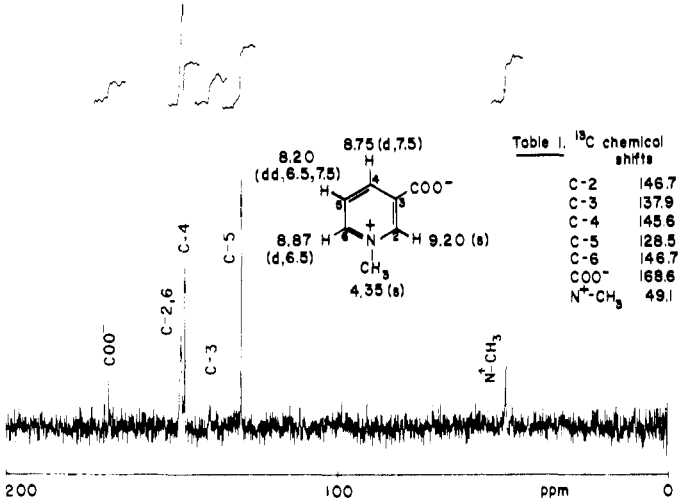


Table I. $^{13}C$ chemical shifts

| | |
|---|---|
| C-2 | 146.7 |
| C-3 | 137.9 |
| C-4 | 145.6 |
| C-5 | 128.5 |
| C-6 | 146.7 |
| COO⁻ | 168.6 |
| $N^+$-$CH_3$ | 49.1 |

**Figure 2.** The proton noise decoupled $^{13}C$ NMR spectrum with 255 $\mu$g of G2 factor in 25 $\mu$L of $D_2O$, using a 1.7-mm microsample insert. The carbon resonances are assigned and are given with the integrated area of each signal. All protonated carbon resonances integrate to approximately one except for the 146.7-ppm (C-2, -6) signal which integrates to two carbons. The insert shows the structure assigned to the G2 factor together with the proton chemical shifts.

phadex G-10, 3 × 36 cm, $H_2O$, gave 45 mg of the crude G2 factor characterized by a UV band at 265 nm. Preparative TLC, $CHCl_3$-MeOH-$H_2O$ (65:65:10), followed by cellulose TLC, MeCN-*i*-PrOH-$H_2O$ (4:3:3), yielded 2 mg of material which was further purified by Sephadex G-15 gel filtration to finally give 50 $\mu$g of pure G2 factor. The entire procedure was repeated four more times to afford a total of ~250 $\mu$g of the bioactive compound which was very polar and insoluble in most organic solvents except $Me_2SO$ and MeOH.

Numerous attempts to measure the mass spectrum (CI-MS, FD-MS, and EI-MS) were unsuccessful. However, satisfactory results were obtained with a sample from one particular 50-$\mu$g batch of G2 factor[5] by high resolution EI-MS,[6] (190 °C, 70 eV) *m/e* 138.0553 (M + H, calcd for 138.0553), 123.0320 (M + H − $CH_3$, calcd 123.0320), 94.0657 (M + H − $CO_2$, calcd 94.0657), 79.0422 ($C_5H_5N$, calcd 79.0422).

The UV in $H_2O$ remained constant above pH 4, $\lambda_{max}$ 265 with a shoulder at 271 nm, but the 271-nm shoulder became more pronounced at pH 2. The FT IR[7] measured as a film (10 $\mu$g) on a KRS-5 plate, 500 transients, is shown in Figure 1; preliminary inspections suggested the presence of some "hydroxylic" function (bands around 3400 $cm^{-1}$),[8] a heteroaromatic nucleus,[9] and three adjacent aromatic hydrogens (780 $cm^{-1}$). The 80-MHz $^1H$ NMR spectrum[10] of 130 $\mu$g, obtained in 1000 transients, was relatively simple and consisted of the following signals (see Figure 2, inset): 4.35 (3 H, s), 8.20 (1 H, dd, $J$ = 6.50 and 7.50 Hz), 8.75 (1 H, d, $J$ = 7.50 Hz), 8.87 (1 H, d, $J$ = 6.50 Hz), and 9.20 ppm (1 H, s).[11] A 20-MHz $^{13}C$ NMR spectrum (Figure 2)[12] of the combined lots of the sample was obtained using a 3-s repetition rate, a 26° flip angle, and 239 400 double precision transients (9 days' accumulation).[14] The peaks at 168.6 and 137.9 ppm were absent from a 3-day accumulation using a 0.819-s repetition rate and 26° flip angle, identifying those peaks as nonprotonated carbons.

The evidence cited above indicated that the G2 factor was *N*-methylnicotinic acid (**1**), a conclusion which was confirmed by comparison with a synthetic sample. *N*-Methylnicotinic acid was first isolated in 1895 from *Trigonella foenum-graecum* ("trigonelline") and has since been isolated from various other sources;[15] however, it has not been associated with any hormonal activity before. Trigonelline is the first natural hormone and natural substance to be chemically characterized from plant or animal sources that regulates cell proliferation by cell arrest in either G1 or G2[3] in complex tissues.

**Acknowledgments.** We are grateful to I. Miura, Columbia University, for $^1H$ NMR measurements (Bruker WP-80), Dr. G. Jordan, Lederle Laboratories, for the FT IR spectrum, and to Dr. H. Kasai for preliminary isolation studies. The studies were supported by NIH Grants CA 11572 (to K.N.) and R01 GM 23232 (to L.S.E.). D.G.L. was supported by NIH Fellowship F32 GM 06464.

## References and Notes

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(2) Evans, L. S.; Van't Hof, J. *Exp. Cell Res.* **1973**, *82*, 471–473. Evans, L. S.; Van't Hof, J. *ibid.* **1974**, *87*, 259–264.
(3) Gap-2 (G2) refers to the stage in the mitotic cycle after cells have doubled their DNA content and before cell division begins. Normally, cells deprived of an energy source will be predominately arrested in G1, after cell division and before DNA synthesis begins, but with the G2 factor a much higher percentage of cells are arrested in G2. See ref 2.
(4) The chromatographic procedures of Scarborough et al. were employed. Scarborough, E.; Armstrong, D. J.; Skoog, F.; Frihart, C. R.; Leonard, N. J. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 3825–3829.
(5) Presumably the batch of G2 factor which gave satisfactory MS results was a mixture of zwitterion **1** and its protonated form, the latter giving the positive MS results.
(6) Measured with a Varian MAT 731.
(7) Measured with a Nicolet 7199 FT IR.
(8) The hydroxylic absorptions around 3400 $cm^{-1}$ are presumably due to the protonated form of **1**.
(9) Katritzky, A. R. "Physical Methods in Heterocyclic Chemistry". Academic Press: New York, 1963; Vol. II, pp 274–303.
(10) Measured with Bruker WP-80.
(11) The coupling constants were determined by computer simulation of the proton spectrum.
(12) Measured on a Varian FT-80A spectrometer, using a 1.7-mm microsample insert in a $^{13}C/^1H$ variable insert probe.
(13) Patt, S. L. Ph.D. Thesis, Harvard University, 1975.
(14) The integrated peak heights greatly facilitated the carbon assignments. Broadening of the three peaks assigned to carbons attached to the nitrogen is due to small residual C–$^{14}N^+$ couplings.
(15) "Merck Index". 9th ed., Merck and Co.: Rahway, N.J., 1976; p 1243.

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