Tumor Inhibitory Agent from *Magnolia grandiflora* (Magnoliaceae) I: Parthenolide

Keyphrases ☐ *Magnolia grandiflora* L.—isolation, identification of parthenolide, antitumor activity ☐ Parthenolide—isolation, identification from *Magnolia grandiflora*, antitumor activity ☐ Antitumor activity—evaluation of parthenolide, isolated and identified from *Magnolia grandiflora*

Sir:

As a result of the continuing search for plants having tumor-inhibiting constituents, it was found that the petroleum ether extract of the leaves and stems of *Magnolia grandiflora* L.[1] showed inhibitory activity toward the human epidermoid carcinoma of the nasopharynx test system (9KB cell culture)[2].

A preliminary examination of the petroleum ether extract revealed one major component. This component was isolated and shown to be the active constituent. The isolation was effected by solvent extraction followed by silica gel G, dry column chromatography. The compound was identified as parthenolide by means of its melting point; mixed melting point; IR, mass spectrometry, NMR, and elemental analyses; and comparison with an authentic sample[3]. The compound demonstrated activity at a dilution level of 2.3×10^1 mcg./ml. Activity in the 9KB cell culture is defined as ED_{50} less than or equal to a dilution of 20 mcg./ml. for plant extracts. The results are expressed as a dose that inhibits growth to 50% of the control growth 3 days after drug addition (1).

The dried leaves and stems (7 kg.) were ground, placed in a Lloyd-type extractor, and exhaustively extracted with petroleum ether (b.p. 40–60°). After removal of the solvent, the residue (160 g.) was treated several times with petroleum ether (b.p. 40–60°). The material obtained from the solution was inactive and, therefore, was discarded. The crystalline insoluble portion (30 g.) was further purified by use of silica gel G, dry column chromatography. Seven grams of the crystalline petroleum ether-insoluble material was placed on a silica gel G column and eluted with dichloromethane–benzene–ethyl acetate (12:24:3). Forty 10-ml. fractions were collected. On the basis of TLC (silica gel G, dichloromethane–benzene–ethyl acetate, 12:24:3), fractions 20–39, which showed a single spot, were combined. The solvent was removed, the residue was dissolved in chloroform and treated with activated charcoal[4], and excess ether was added after evaporation of most of the solvent. A crystalline precipitate (2.9 g.) occurred which was identified as parthenolide (2).

[1] The plant was collected on the campus of the University of Arizona, Tucson, Ariz., in January 1969. Identification was confirmed by Robert J. Barr, College of Pharmacy, and Dr. Charles Mason, Botany Department, University of Arizona. A reference specimen was also deposited in the University of Arizona Herbarium.
[2] Drug Evaluation Branch, Drug Research and Development, Chemotherapy, National Cancer Institute.
[3] The authors are indebted to Dr. B. S. Joshi, Ciba of India Ltd., Goregaon Bombay, India, for providing the authentic sample of parthenolide.
[4] Norite.

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Simple Method for Portal Vein Infusion in the Rat

Keyphrases ☐ Portal vein infusion method—used to study first-pass effect in rats ☐ First-pass effect—studied using simple method for portal vein infusion, rats ☐ Absorption, first-pass effect—studied using simple method for portal vein infusion, rats

Sir:

The rate and extent of drug absorption into the systemic circulation have been estimated by pharmacokinetic analysis of plasma concentration–time data or urinary excretion data (1, 2). The percent of absorption can be assessed by comparison of the relative areas under the plasma concentration–time curves after oral and intravenous administration. This method is based on the presumptions that the distribution and elimination of a drug may be expressed in terms of first-order kinetics within the dose range studied and that the parameters of the distribution and elimination remain constant after administering the same quantity of drug by different routes. Thus, the resultant areas are independent of the route of administration and proportional to the dose even when given by different routes. However, it was recently shown that the areas under the blood level–time curves for aspirin (3) and lidocaine (4) after infusion into a peripheral vein were considerably greater as compared with results observed after infusion of an equal dose into the portal vein in dogs. The reduction in area under the blood level–time curves after portal vein infusion has been attributed to a significant degree of metabolism of the drugs during the first passage through the liver.

In this communication, we report a simple method for portal vein infusion in the rat to study the first-pass

effect, using propranolol as a test compound. The method consists of cannulation into the pyloric vein. A rat weighing 200–240 g. was anesthetized lightly with ether at suitable intervals, and the abdomen was opened through a midline incision. The pyloric vein was exposed by pushing aside the duodenum of the intestine, and a minimal amount of connective tissue was removed from the pyloric vein to facilitate ligation. The pyloric vein was then ligated approximately 0.7 cm. distal to the junction of the pyloric vein and hepatic portal vein. A cannula (polyethylene tubing, 0.04-cm. i.d., 0.06-cm. o.d., 7-cm. length), filled with heparin solution (200 units/ml.) and closed with an arterial clamp at the other end, was inserted into the pyloric vein, approximately 0.2 cm. proximal to the ligature, after careful venous puncture using a needle point. The cannula was gently guided upward into the portal vein so that its tip projected several millimeters into the vein (Fig. 1). The cannula was secured by ligation with nylon suture. A syringe was attached to the end of the cannula, and it was then confirmed that the portal blood could be detected in the cannula with a slight suction. The abdominal incision was closed, and the syringe was replaced by another syringe containing infused solution. After the end of the infusion experiment, it is desirable to inject methylene blue solution at an appropriate concentration into the cannula and to observe the liver stained with the dye in order to check for lack of leakage during infusion.

Propranolol was administered to rats by rapid infusion into the femoral vein or by constant infusion into the hepatic portal vein during 50 min. using an infusion pump. Blood samples (0.1–0.2 ml.) were taken at various intervals through a cannula inserted into the femoral artery and were diluted with 1.0 ml. of water prior to extraction. The propranolol concentrations in whole blood were determined spectrophotofluorometrically by a minor modification of the method of Shand *et al.* (5). The fluorescence of propranolol in 0.1 *N* HCl was measured in a spectrophotofluorometer[1] (maximum excitation 295 nm., maximum emission 360 nm.). An equal volume of blood to sampled blood was transfused each time by an intravenous infusion. The blood for transfusion was taken from another rat of the same strain.

The blood concentration–time curve after intravenous administration was described in all rats by a biexponential equation of the form $C = Ae^{-\alpha t} + Be^{-\beta t}$. Table I lists the mean areas under the blood concentration–time curves after intravenous and portal vein infusion. The data show that the mean areas under the blood concentration–time curves after portal vein infusion were found to be 7.8 (2.5 mg./kg.) and 90.9% (12.5 mg./kg.) of the mean areas of the corresponding intravenous infusion curves. Although the area under the blood concentration–time curve after intravenous administration was directly proportional to the dose, the mean area obtained after portal vein infusion at a dose of 2.5 mg./kg. was less than 10% of the mean area of intravenous infusion at an equal dose. Shand *et al.* (5) reported that, in the same human subjects, propranolol reaching the systemic circulation was estimated to be 16–60% of an oral dose from the ratio of the areas under the plasma concentration curves after oral and intravenous administration. After oral administration of doses less than 30 mg., only trace amounts of propranolol were detected in the systemic circulation in six human subjects, while the area under the blood concentration–time curve was linearly related to dose with oral doses exceeding 40 mg. (6). The results in rats show that the clearance of propranolol from the portal circulation during its first passage through the liver can explain the significant reduction of the area under the curve at the lower dose.

Table I—Mean Areas under Blood Concentration–Time Curves after Rapid Intravenous and Constant Portal Vein Infusion of Propranolol

Administration Route[a]	Dose, mg./kg.	Number of Rats	Area Mean ± *SE*, ng. min./ml.	Relative Area
Intravenous	2.5	3	52746 ± 3937[b]	1.0
	12.5	3	260546 ± 19245[b]	4.9[c]
Intraportal	2.5	5	4129 ± 750[d]	0.078[e]
	12.5	5	236837 ± 21946[d]	0.909[e]

[a] Intravenous doses were given within 30 sec. into the femoral vein, and intraportal doses were given at a constant rate during 50 min. into the hepatic portal vein. [b] Calculated as $(A/\alpha + B/\beta)$. [c] Compared with the area for an intravenous dose of 2.5 mg./kg. [d] Calculated using the trapezoidal rule. The area for the tail end after the last sampling at a dose of 12.5 mg./kg. was calculated by C_t/β, where C_t is the blood concentration at time t, and the rate constant β was estimated from the terminal slope of a semilogarithmic plot of the blood concentrations of propranolol after the end of infusion. [e] Compared with the areas for the corresponding intravenous doses. Significantly different at a dose of 2.5 mg./kg. ($p < 0.001$) and not significantly different at a dose of 12.5 mg./kg. ($p < 0.4$).

Administration of a drug into the portal vein blood has been attempted only in the dog to study differences in areas under blood level–time curves as a function of route of administration (3, 4). In these reports, a cannula was introduced into the hepatic portal vein through a branch of the splenic vein (3) or a tributary of the superior mesenteric vein (4). The method for sampling portal vein blood presented by Pelzmann and Havemeyer (7) could be used for administering a drug into

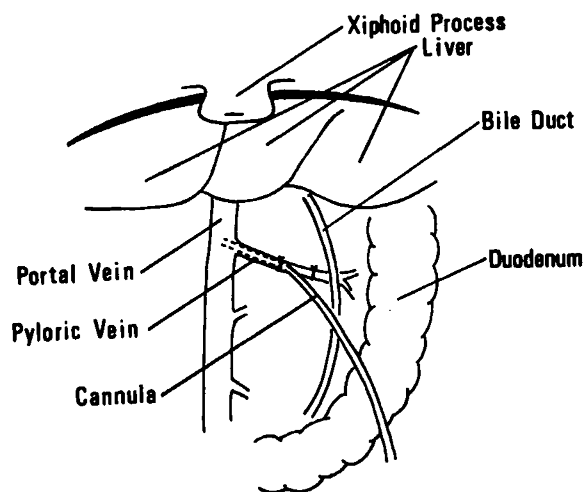

Figure 1—*Representation of pyloric vein cannulation. Mesenteries were retracted to show the cannulation.*

[1] Hitachi model 203.

the portal vein of the rat. However, the portal vein infusion using the pyloric vein cannulation overcomes several disadvantages inherent in the use of direct cannulation into the portal vein, since the latter surgery is delicate, requiring speed and accuracy to prevent excessive engorgement of the intestinal veins and acute loss of blood in the liver. The pyloric vein was selected because of its accessibility and a suitable branch of the hepatic portal vein to facilitate cannulation. The method for portal vein infusion in this communication is simple and practical as compared with that in the dog. This procedure in the rat has proven useful in drug absorption and metabolism studies. Details of these studies will be reported elsewhere.

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BOOKS

REVIEWS

Amino-Acids, Peptides, and Proteins. A Specialist Periodical Report. Volume 3. G. T. Young, Senior Reporter. The Chemical Society, London, WIV OBN, England, 1971. xiv + 379 pp. 14.5 × 22 cm. Price $6.00.

This third volume continues the annual review of the literature in the field of amino acids, peptides, and proteins, covering about 2300 papers published mostly in 1970. Under the editorship of G. T. Young from Oxford (who is called "senior reporter" in the Chemical Society's terminology), a group of 15 "reporters" summarize the developments during 1970 in 5 chapters, giving brief abstracts of the papers or mere one line notations, depending on the significance ascribed to the publication. Chapter 1 by B. W. Bycroft covers amino acids (30 pages) in a rather readable fashion. Chapter 2, "Structural Investigation of Peptides and Proteins," takes up most of the space (188 pages). It involves a number of contributors and includes extensive coverage of the work on sequencing and X-ray crystallography of proteins. Chapter 3, "Peptide Synthesis" (57 pages), by J. H. Jones opens with some statistics to document the reporter's contention that essentially all the important news on this topic can be covered by monitoring 25 primary journals. The last two chapters discuss "Peptides with Structural Features not Typical of Proteins" (47 pages) and "Metal Derivatives of Amino-acids, Peptides, and Protein" (36 pages), the latter covering the two-year period of 1969–1970.

The volume is in keeping with the high standards which one has come to expect of the "Specialist Periodical Reports." The reviewer is not expert enough to judge whether the coverage is complete but at least did not notice any major omissions. As is almost unavoidable in a compilation of a huge amount of material like this, occasional errors do creep in, although the number seems to be quite small. One picky little example (as the customary documentation that the reviewer has read the book): A table on page 13 lists among α-amino acids which have been synthesized for the first time, 5-fluorotryptophan with a 1969 reference, a compound which this reviewer has bought as a catalog item from a commercial supplier as far back as 1963. This leads me to what I consider the main shortcoming of this type of publication. The information in the literature is strictly "reported" including many of the claims of the original authors, leaving essentially all the evaluation of the work to the reader. Since many claims take on a stronger flair of truth if they are repeated by third parties, this means that unless the reader goes back to the original literature, he may be left with some conclusions which are not as soundly based as he may think. While this referee would prefer a more critical review of the literature, the contributors usually stick to their assigned role as impartial reporters, with a few notable exceptions (*e.g.*, Jones on pages 247 and 248).

This difference in taste notwithstanding, the reviewer feels that this volume belongs on the bookshelf of every chemist and medicinal chemist who has anything to do with amino acids or their polymers since it is one of the most comprehensive sources of information in this field.

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Food Chemicals Codex, Second Edition. Prepared by the Committee on Specifications, Food Chemicals Codex, of the Committee on Food Protection, National Research Council, National Academy of Sciences, 2101 Constitution Ave., N.W., Washington, DC 20418, 1972. 1039 pp. 15 × 23 cm. Price $20.00.

This edition, slightly larger than the first, contains 639 monographs. Monographs are provided for chemicals added directly to food to perform some desired function as well as substances