Beta-Solamarine: Tumor Inhibitor Isolated from Solanum dulcamara

Abstract. *An alcoholic extract of* Solanum dulcamara *L., a plant widely used in folk medicine for treating cancers and warts, shows tumor-inhibitory activity against Sarcoma 180 in mice. Systematic fractionation of the extract has led to isolation and characterization of β-solamarine as an active principle.*

Solanum dulcamara L. ("bittersweet" or "woody nightshade") has been used to treat cancers, tumors, and warts from the time of Galen (circa A.D. 180) (*1*), and references to its use have appeared in the literature of the United States (*2*), Chile (*3*), Italy (*4*), Spain (*5*), France (*6*), Germany (*7*), England (*8*), Denmark (*9*), Iceland (*10*), and China (*11*).

During our search for tumor inhibitors from plant sources, alcoholic extracts of dried *Solanum dulcamara* from New York (two separate samples, one of root and rhizome, and the other of stem, leaf, flower, and fruit), from Wisconsin (fruit, leaf, and stem), and from Spain (stem, leaf, flower, and fruit) each showed significant inhibitory activity when tested in mice against Sarcoma 180 (*12–14*). We report here the fractionation of an active extract and the isolation and characterization of a tumor-inhibitory principle which is identified as β-solamarine.

Solvent partition of the alcoholic extract (*A* in Fig. 1) of the dried Wisconsin sample between water and chloroform yielded an active (Table 1) aqueous phase (*B*) free of chlorophylls and fatty materials. The aqueous phase was made alkaline with sodium bicarbonate and extracted with butanol, whereupon activity was concentrated in the butanol layer (*E*). Preliminary investigation of the butanol-solubles (*E*) by Craig countercurrent distribution led to correlation of activity with a specific spot upon thin-layer chromatography and showed that the active material was alkaloidal. A concentrated alkaloidal fraction was obtained from a solution of the butanol-soluble residue in 4 percent acetic acid by reprecipitation with ammonium hydroxide. Partition chromatography of the reprecipitated alkaloids on alumina (by means of butanol saturated with water as eluant) and analysis of the fractions by thin-layer chromatography revealed at least eight components reactive to Dragendorf's reagent.

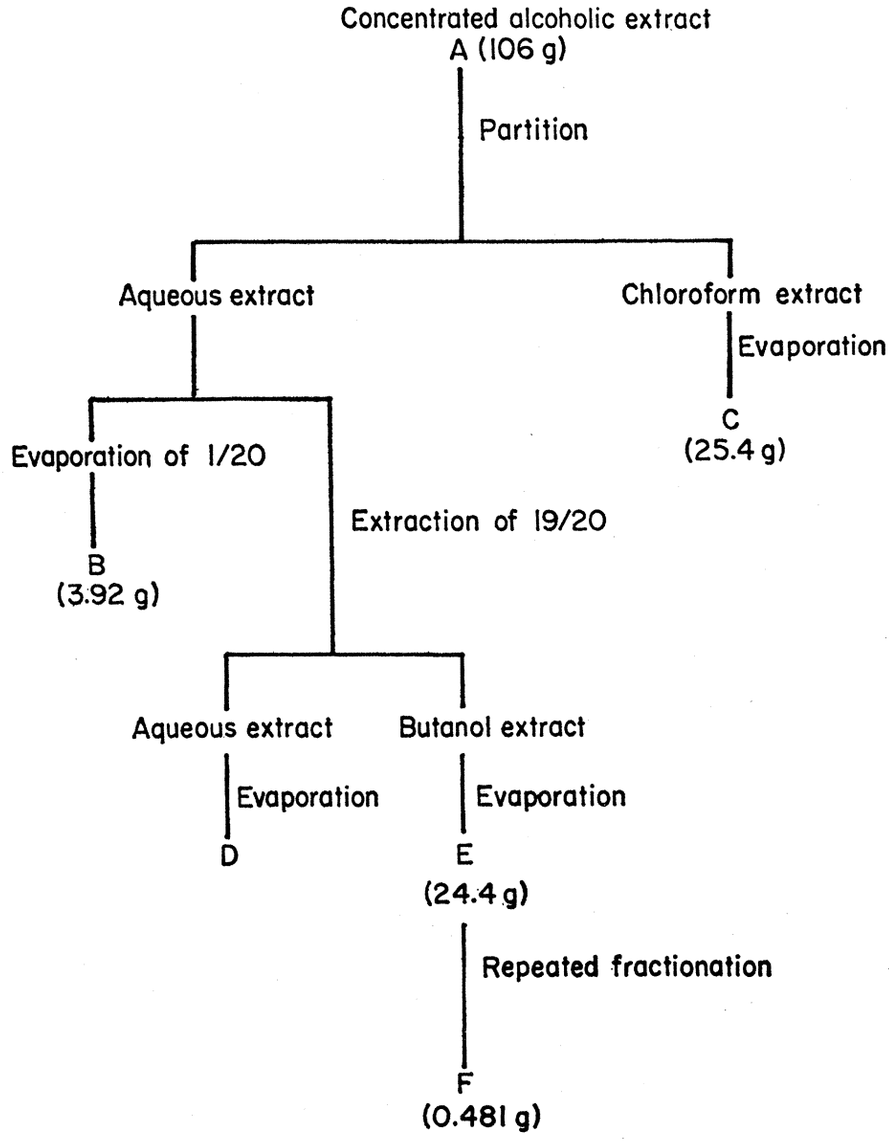

Fig. 1. Flow sheet for fractionation of tumor-inhibitory extract from *Solanum dulcamara* L.

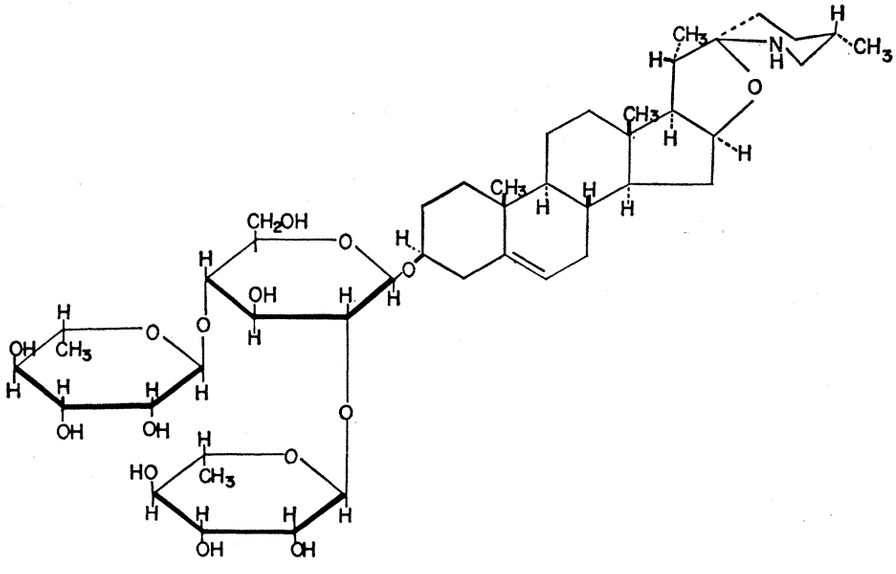

Fig. 2. Structure of β-solamarine.

Table 1. Activity of fractions from *Solanum dulcamara* against Sarcoma 180 in mice.

Dose (mg/kg)	Survivors	Change in animal weight (g)*	Tumor weight in mg (test/control)
	Fraction A		
266	0/4		
177	1/4	−4.1	275/935
118	4/4	−1.9	346/935
	Fraction B		
300	3/4	−4.5	0/1015
150	4/4	−4.4	356/938
75	4/4	0.6	629/938
37.5	3/4	−1.4	826/938
	Fraction C		
200	3/4	−1.7	348/804
100	4/4	−0.4	543/804
50	4/4	−1.2	791/804
	Fraction D		
300	4/4	1.9	1571/867
150	4/4	0.1	1203/867
75	4/4	−1.0	1349/867
	Fraction E		
300	0/4		
150	0/4		
75	3/4	−3.2	180/804
	Fraction F		
60	0/4		
30	4/4	−1.8	274/1285
15	4/4	−1.6	806/1285

* The difference, in grams, between weights of test and control animals.

The major component of one of the fractions was separated from the remainder of the material by countercurrent distribution (upper phase, 50 percent butanol in ethyl acetate, and lower phase, *p*H 4 citrate-phosphate buffer) and further alumina chromatography. The compound (*F*) separated from methanol-acetone as a microcrystalline solid which melted at 267° to 270°C (decomp.) and showed a specific rotation ($[\alpha]_D^{28}$) of −81 deg in pyridine solution. There was no depression of the melting point on admixture with authentic β-solamarine (m.p. 256° to 259°C) [literature m.p. 275° to 277°C (softens, 270°C), $[\alpha]_D^{20}$ = −85.6 deg (pyridine) (*15*)] and the infrared spectra of the respective samples (KBr pellets) could be superimposed. The respective samples showed identical R_F values upon thin-layer chromatography on silica gel with butanol, acetic acid, and water (4 : 1 : 5, upper phase); ethanol, chloroform, and 1 percent ammonium hydroxide solution (2 : 2 : 1, lower phase); and ethyl acetate, pyridine, and water (3 : 1 : 3, upper phase). Hydrolysis of our sample with methanolic 1*N* hydrochloric acid and recrystallization of the resulting aglycone from acetone gave a product of m.p. 236° to 238°C and $[\alpha]_D^{29}$ −34 deg (methanol). The aglycone was characterized as Δ^5-tomatidenol [literature m.p. 238° to 240°C and $[\alpha]_D$ −37.9 deg (methanol) (*15*)] by mixed melting point and mixed thin-layer chromatographic comparison with an authentic sample (m.p. 237° to 239°C).

β-Solamarine has previously been isolated from *Solanum dulcamara* L. (*15*) and has been assigned the steroid alkaloid glycoside structure shown in Fig. 2 by P. M. Boll (*16*). The tumor-inhibitory activity of steroid alkaloid glycosides does not appear to have been reported previously.

S. Morris Kupchan
Stanley J. Barboutis
John R. Knox
Cesar A. Lau Cam
Department of Pharmaceutical Chemistry, University of Wisconsin, Madison

References and Notes

1. L. Israelson, "Die Materia Medica des Klaudios Galenos" [dissertation, Jurjew (Dorpat), Univ. Dorpat, Schnakenburg, 1894]; G. Urdang, G. D. Goldat, D. Queller, G. Sonnedecker, "An Examination of Old Literature (Especially Herbals) for Drugs with Supposed Effects on Cancer" [Report to NIH on contract No. C-2089 (C) with the Univ. of Wisconsin, 1956].
2. S. Henry, *American Medical Family Herbal* (S. Henry, New York, 1814); N. Chapman, *Elements of Therapeutics and Materia Medica* (Carey and Lea, Philadelphia, ed. 4, 1825); O. P. Brown, *The Complete Herbalist*, (O. P. Brown, Jersey City, N.J., 1867).
3. J. Zin, *La Salud por Medio de las Plantas Medicinales por un Sacerdote Selesiano* (La Gratitud Nacional, Santiago de Chile, ed. 5, rev., 1930).
4. G. Scotti, *Flora Medica della Provincia di Como* (C. Franchi, Como, 1872).
5. A. Fleury de la Roche (trans. by F. Salas), *Las Plantas Bienhechoras* (Iberia, Barcelona, 1931).
6. M. Fouquet, *Les Remedes Charitables de Madame Fouquet* (J. Certe, Lyon, ed. 3, 1685).
7. J. Wolff, *Die Lehre von der Krebskrankheit* (G. Fischer, Jena, 1914); L. Kroeber, *Das Neuzeitliche Kräuterbuch* (Hippokrates-Verlag, Stuttgart-Leipzig, 1934, 1935).
8. W. Coles, *Adam in Eden, or Nature's Paradise* (J. Streater, London, 1657); N. Culpeper, *The English Physitian Enlarged* (G. Sawbridge, London, 1681).
9. C. Molbeck, Ed., *Henrik Harpestrengs Danske Laegebog* (H. H. Thiele, Copenhagen, 1826).
10. H. Larsen, *An Old Icelandic Medical Miscellany* (J. Dybwad, Oslo, 1931).
11. G. A. Stuart, *Chinese Materia Medica* (Am. Presbyterian Mission Press, Shanghai, 1911).
12. For the plant samples from New York and from Spain, we thank Dr. Robert E. Perdue, U.S. Dept. of Agriculture, Beltsville, Md.; they were delivered in accordance with the program developed with the USDA by the Cancer Chemotherapy National Service Center (CCNSC), NIH.
13. Evaluation of assay results by CCNSC on a statistical basis in sequential testing is such that a material is considered active if it causes reduction of tumor weight to 42 percent or less; for further details see *Cancer Chemotherap. Rep.* **25**, 1 (1962).
14. Assays were performed by the Wisconsin Alumni Research Foundation under the auspices of CCNSC.
15. P. M. Boll, *Acta Chem. Scand.* **16**, 1819 (1962).
16. ———, *ibid.* **17**, 1852 (1963).
17. This communication is part 13 in the series entitled "Tumor Inhibitors"; part 12 is by S. M. Kupchan, J. M. Cassady, J. Bailey, J. R. Knox, *J. Pharm. Sci.* **54**, 1703 (1965). Work supported by the Nat. Cancer Inst. (grant CA-04500), the Amer. Cancer Soc. (T-275), and the CCNSC (contract PH-43-64-551). We thank Dr. J. L. Hartwell, CCNSC, for the references on medicinal uses of *Solanum dulcamara* L., Prof. P. M. Boll for the authentic β-solamarine, and Dr. Y. Sato, NIAMD, for the authentic Δ^5-tomatidenol.

7 October 1965

Sex Chromatin of Cone Cells of Human Retina

Abstract. *Each retinal cone cell of the female contains a sex-chromatin body not present in comparable material from the male. Therefore the lack of detectable "patch" formation in female heterozygotes for red color-blindness (as expected from the X-inactivation hypothesis) cannot be attributed to failure of Barr-body formation.*

Most cells of the mammalian female are functionally mosaic for the X-linked genes. This is the result of heteropyknosis of one of the two X-chromosomes in each somatic cell of the female. The evidence for this was originally derived from the finding of a deeply stained body (sex-chromatin mass) in the nuclei of cells of female cats; this body is not present in males (*1*). Moore and Barr (*2*) later suggested that the heteropyknosis of the X-chromosome known to occur in certain insects (*3*) might also occur in mammalian cells, causing the stainable nuclear mass. This suggestion was shown to be essentially correct when it was demonstrated that only one of the two X-chromosomes was condensed at prophase and thus was probably responsible for the sex-chromatin mass (*4*). The cytologic data were substantiated when experiments with tritiated thymidine tracer revealed that one of the X-chromosomes in mammals is labeled asynchronously with respect to the other (*5*).

Under certain circumstances condensation of an X-chromosome in cells of the female does not occur. For example, neither oogonia nor oocytes show a heteropyknotic X-chromosome (*6*). In somatic cells, the condensed X-chromosome is extended during some phase of the mitotic cycle, presumably when replication of DNA takes place (*7*). In the fertilized ovum, the female zygote shows no condensed X until some time after blastulation and im-