formed was collected by filtration and dried to yield 0.8 g. (63.6%) of 6(or 7)-(1-aziridinyl)-5,8-isoquinolinedione (IX), m.p. 143–145°; ν_{max} (KBr) 1638 and 1670 cm.$^{-1}$ (C=O); λ_{max} (CH_3CN) 318, 258, and 236 mμ (log ε 3.77, 4.16, and 4.12). This compound is irritating and should be handled with care. It gives a positive Craven test.[17]

Anal. Calcd. for $C_{11}H_8N_2O_2$: C, 66.00; H, 4.03; N, 13.99. Found: C, 65.94; H, 4.12; N, 13.98.

Acknowledgment.—The authors wish to thank Dr. Robert J. Rutman and his staff for the biological results.

Tumor Inhibitors. IV.[1] Apocannoside and Cymarin, the Cytotoxic Principles of *Apocynum cannabinum* L.[2]

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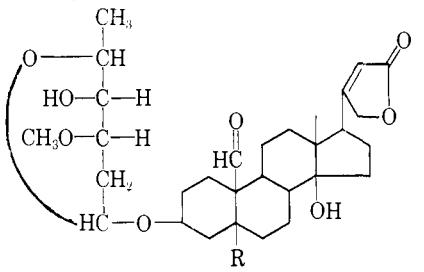
In the course of a continuing screening program for tumor inhibitors from plant sources, an alcoholic extract of the roots of *Apocynum cannabinum* L. was found to have significant inhibitory activity against human carcinoma of the nasopharynx carried in cell culture (KB).[4-6] We report herein the fractionation of the active extract and the isolation and characterization of the active principles apocannoside (I) and cymarin (II).



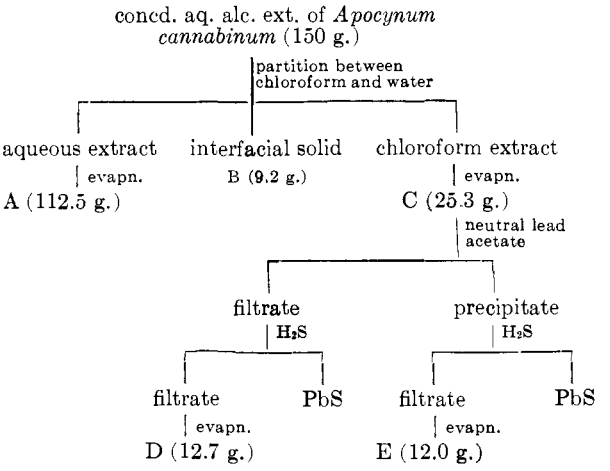
I, R = H
II, R = OH

Partition of the concentrated aqueous alcoholic extract of the roots of *A. cannabinum* between water and chloroform resulted in a concentration of the activity (Table I) in the chloroform phase (C, Scheme I). The material recovered from the chloroform layer was dissolved in methanol and treated with excess 10% methanolic neutral lead acetate solution. Removal of the precipitate by centrifugation and of the excess lead with hydrogen sulfide yielded a still more active extract (D).

TABLE I
CYTOTOXICITY OF FRACTIONS FROM *A. cannabinum*

Fraction	ED_{50}, γ/ml.	Fraction	ED_{50}, γ/ml.
A	3.0	J	45
B	1.5	K	26
C	0.55	L	0.062
D	0.6	M	0.021
E	30.4	N	0.45
F	>100	O	5.0
G	>100	P	0.037, 0.098
H	>100	Q	30
I	>100	R	0.0039, 0.016

SCHEME I
FRACTIONATION OF THE CYTOTOXIC PRINCIPLES OF *Apocynum cannabinum*



Further fractionation of fraction D was effected by adsorption chromatography on a silicic acid–Celite 545 column, whereby the activity was concentrated into two yellow oily fractions (L and M). Fraction L was crystallized from methanol–ether to yield apocannoside (I), characterized by comparison of the physical properties of the glycoside[7] and of its acetate[8] with reported values. Fraction M was crystallized from methanol–ether to yield cymarin (II), characterized by comparison of the physical properties of the glycoside[9] and of its aglycone, strophanthidin,[10] with reported values.

The biological testing data (Table I)[6] indicate that apocannoside and cymarin are chiefly responsible for the cytotoxicity of the extract of *A. cannabinum*. The cytotoxicity of the two glycosides is sufficiently high to warrant scheduling the compounds for testing in a variety of *in vivo* tumor systems. It is noteworthy that, although a survey of the literature has revealed

(1) Part III in the series: S. M. Kupchan, R. W. Doskotch, and P. W. Vanevenhoven, *J. Pharm. Sci.*, **53**, 343 (1964).

(2) This investigation was supported by grants from the National Cancer Institute (PHS Research Grant No. CA-04500-06) and the American Cancer Society (T-275). R. J. H. gratefully acknowledges receipt of a Wellcome Research Travel grant awarded by the Wellcome Trust.

(3) Author to whom inquiries should be addressed.

(4) Our original plant sample was gathered in Maryland in July, 1960. We acknowledge with thanks the receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture, Beltsville, Md., in accordance with the program developed with the USDA by the Cancer Chemotherapy National Service Center.

(5) We thank Dr. Jonathan L. Hartwell of the National Cancer Institute for informing us of his independent observation of cytotoxicity toward KB cell culture of an aqueous alcoholic extract of *Apocynum cannabinum* L. ("Black Indian Hemp Root") available commercially from the Meer Corp., New York, N. Y., and for placing at our disposal a supply of this extract for fractionation studies.

(6) The evaluation of the KB assay results by the Cancer Chemotherapy National Service Center in sequential testing is such that a purified compound is considered active if the average ED_{50} of two tests ≦ 4 γ/ml. and if this result is reproducible by a second screener. In the event that a compound has an ED_{50} of < 1 γ/ml. in the first test, the second sequential test is omitted and it is submitted to a second screener for confirmation. The procedures were described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

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(8) C. H. Trabert, *Arzneimittel-Forsch.*, **10**, 197 (1960).

(9) W. A. Jacobs and A. Hofmann, *J. Biol. Chem.*, **67**, 609 (1926).

(10) N. K. Sen, J. K. Chakravarti, W. Kreis, C. Tamm, and T. Reichstein, *Helv. Chim. Acta*, **40**, 588 (1957).

no reference to the use of *A. cannabinum* for the treatment of cancer, a milky bitter juice obtained from the fresh plant has been used against warts and condylomas.[11]

We have noted with considerable interest the recent report that, of more than 150 steroids tested for cytotoxic activity against KB, the most active compounds all contain an α,β-unsaturated lactone ring.[12] Studies are in progress in these laboratories which are aimed at evaluation of the importance of the γ-lactone ring, the 19-oxo function, the glycosidic moiety, and other structural features to the cytotoxicity of cardenolide derivatives.

Experimental[13]

Separation into Main Fractions.—The aqueous alcoholic extract (150 g.) of the roots of *Apocynum cannabinum* (Meer Corp.) was partitioned between water (500 ml.) and four 250-ml. portions of chloroform. The resulting aqueous layer was freeze-dried to yield 112.5 g. of aqueous extract (A). The chloroform solution, after drying over anhydrous sodium sulfate, was evaporated under reduced pressure to yield 25.3 g. of chloroform solubles (C). The interfacial insolubles after drying yielded 9.2 g. of solid (B).

The chloroform-soluble fraction (C) was dissolved in methanol (250 ml.) and treated with excess 10% methanolic lead acetate solution. The precipitate was removed by centrifugation and the centrifugate was freed from excess lead by treatment with hydrogen sulfide. The precipitate, after resuspending in methanol (250 ml.), was regenerated by separate treatment with hydrogen sulfide. Evaporation of the two solutions under reduced pressure yielded 12.7 g. of the nonprecipitated (D) and 12.0 g. of precipitated material (E). (See Scheme I for flow sheet.)

Isolation of the Glycosides.—The nonprecipitated material was further fractionated by adsorption chromatography on a silicic acid (Mallinckrodt)–Celite 545 (Johns Manville) (3:1, 500 g.) column, 40 × 5 cm. Fraction D (12.7 g.), dissolved in chloroform (150 ml.), was added to the column and washed on with a further 100 ml. of chloroform. The column was then eluted with 1% methanol in chloroform to yield a yellow waxy solid (F, 2.60 g.) and a yellow oil (G, 0.72 g.). Elution was continued with 3% methanol in chloroform to yield a yellow crystalline solid (H, 3.75 g.), a yellow waxy solid (I, 0.34 g.), and another yellow crystalline material (J, 0.86 g.). The solvent was changed to 5% methanol in chloroform to yield a brown oil (K, 0.50 g.) and two yellow oil fractions (L, 0.69 g., and M, 0.76 g.). The material remaining on the column was removed using methanol to yield a fawn solid (N, 1.19 g.).

Fraction L (0.69 g.) was crystallized from methanol–ether to yield 230 mg. of colorless needles of apocannoside (P), m.p. 134–137° (lit.[7] m.p. 122–132°); $[\alpha]^{23}D$ −8° (*c* 0.91, $CHCl_3$); λ_{max}^{alc} 216 mμ (ε 12,600); $\lambda_{max}^{CHCl_3}$ 2.83, 3.41, 5.63, 5.76, 5.84, and 6.20 μ. The acetate was obtained from acetone–ether as colorless prisms, m.p. 182–184° (lit.[8] m.p. 175–185°), $[\alpha]^{24}D$ +4° (*c* 1.04, $CHCl_3$).

The residual oil (445 mg.) obtained from the above crystallization of apocannoside was rechromatographed on a silicic acid (Mallinckrodt)–Celite 545 (Johns-Manville) (3:1, 12 g.) column as previously described. The fraction containing apocannoside was rechromatographed on silica gel thin layer plates using 10% methanol in chloroform as solvent. The apocannoside band (R_f 0.60–0.65) was removed and eluted with methanol to yield 36 mg. of crystalline apocannoside. The remaining silica gel was also washed with methanol and the residue after removal of the methanol was combined with the apocannoside-free material obtained from the column, yielding residual apocannoside-free oil (O, 328 mg.).

Fraction M (0.76 g.) was crystallized from methanol–ether, yielding 255 mg. of colorless needles of cymarin (R), m.p. 143–144° (lit.[9] 138–148°); $[\alpha]^{23}D$ +38° (*c* 1.07, $CHCl_3$); λ_{max}^{alc} 216 mμ (ε 9800); $\lambda_{max}^{CHCl_3}$ 2.87, 3.40, 5.62, 5.76, 5.85, and 6.19 μ.

Acid hydrolysis yielded aglycone which was obtained from methanol–water as colorless prisms, m.p. 150–152°, $[\alpha]^{24}D$ +42° (*c* 1.2, MeOH). This sample also had the same R_f as that of an authentic sample of strophanthidin on thin layer chromatography on silica gel using 10% methanol in chloroform. The infrared spectrum in Nujol was essentially superimposable with that of the authentic sample of strophanthidin.

The residual oil (489 mg.) obtained from the above crystallization of cymarin was rechromatographed on a silicic acid (Mallinckrodt)–Celite 545 (Johns-Manville) (3:1, 12 g.) column as previously described. The fraction containing cymarin was chromatographed on silica gel thin layer plates using 10% methanol in chloroform as solvent. The cymarin band (R_f 0.40–0.48) was removed and eluted with methanol to yield 162 mg. of crystalline cymarin. The remaining silica gel was also washed with methanol and the residue after removal of the methanol was combined with the cymarin-free material obtained from the column, yielding residual cymarin-free oil (Q, 233 mg.).

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(12) J. E. Pike, J. E. Grady, J. S. Evans, and C. G. Smith, *J. Med. Chem.*, **7**, 348 (1964).

(13) Melting points have been corrected for stem exposure. Values of $[\alpha]D$ have been approximated to the nearest degree. Infrared spectra were determined on a Beckman infrared 5A spectrophotometer. Ultraviolet absorption spectra were determined in 95% ethanol on a Beckman DK 2A recording spectrophotometer.

The Oral Progestational Activity of the 3-Ketals of Certain 17-Acetoxy- and 17-Alkylprogesterones

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In the course of our studies[1] in the 17-alkylprogesterone series we observed that in most instances the 3-ethylene ketal derivative was at least as effective in the oral Clauberg assay as the parent ketone and in some instances even showed enhanced activity.[2] To investigate further this interesting observation we prepared the 3-ketals of a variety of known active 17-acetoxyprogesterones, namely 17-acetoxyprogesterone[3] and its 6α-methyl,[5] 6-dehydro,[6,7] 6-dehydro-6-methyl,[8] and 6-chloro-6-dehydro[9] derivatives. In addition, the 3-ketals of 6-dehydro-17-ethylprogesterone[10] and its 6-chloro derivative[10] were prepared.

The various ketals (Table I) were obtained by direct ketalization, according to the usual technique,[11] of

(1) M. J. Weiss, R. E. Schaub, G. R. Allen, Jr., J. F. Poletto, C. Pidacks, R. B. Conrow, and C. J. Coscia, *Tetrahedron*, **20**, 357 (1964).

(2) In a previous report from this laboratory [W. S. Allen, H. M. Kissman, S. Mauer, I. Ringler, and M. J. Weiss, *J. Med. Pharm. Chem.*, **5**, 133 (1962)] we noted the glucocorticoid activity of various 20-ketalized corticoids.

(3) Although the preparation of the 3-ethylene ketal of 17-acetoxyprogesterone has been reported,[4] no biological activity was noted.

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(9) H. J. Ringold, E. Batres, A. Bowers, J. Edwards, and J. Zderic, *ibid.*, **81**, 3485 (1959).

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