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Note

Synthesis of 8-*O*-(α-L-arabinofuranosyl)-β-peltatin-A*

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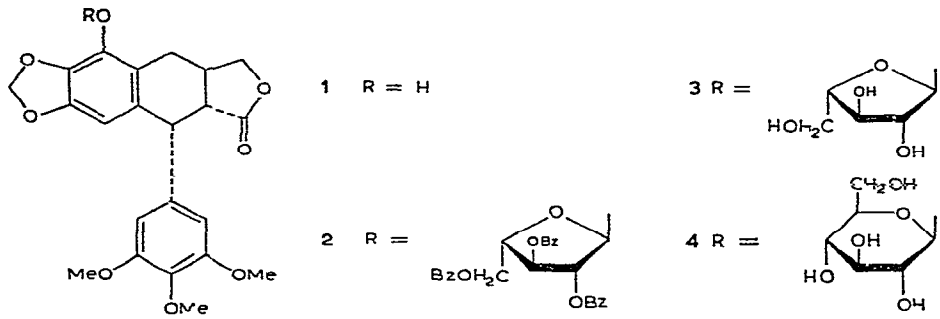
As part of a new approach in the chemotherapy of cancer[1] it is proposed to achieve increased selectivity of chemotherapeutic measures against malignant tumors by administering relatively non-toxic, transport-forms of cancerostatic agents simultaneously with a foreign enzyme, active in the acidic pH range (pH 6 5–6 8) of the tumor, that is capable of yielding in the tumor area the active form of the inhibitory moiety Because of the negligible or low activity of the enzyme in the neutral pH range, toxification of the transport-form in normal tissues (pH 7 3–7.4) should be virtually nil

Hitherto, we have investigated this concept with several synthetic transport-forms of alkylating agents[2–5]. In these studies, evidence was obtained for the first time that transport-forms can be selectively cleaved in tumor tissues to yield their toxic moiety[1]. In particular, it has been shown that α-L-arabinofuranosides are effective as transport-forms, because they are not endogeneously cleaved in the animal (mouse) After injection of *p*-nitrophenyl α-L-arabinofuranoside, no *p*-nitrophenol was found in the urine of the animals By contrast, *p*-nitrophenyl β-D-glucopyranoside and -xylopyranoside, for example, are known to undergo endogenous cleavage[6] Another advantage of the α-L-arabinofuranosides is the availability of an α-L-arabinofuranosidase (*Aspergillus niger* K 1)[7], showing a desirable pH dependence of its activity in relation to many substrates[1] It was thus of interest to test a transport-form of a potent mitosis inhibitor For this purpose we selected compound **1**, a lignan of podophyllin, whose strong antimitotic and cancerostatic effects have been described[8–10] We now report the first synthesis of its α-L-arabinofuranoside (**3**) Only one naturally occurring glycoside of β-peltatin-A (**1**) is known thus far, namely 8-*O*-(β-D-glucopyranosyl)-β-peltatin-A[11] (**4**), isolated from *Podophyllum peltatum* L.

The starting material was **1**, isolated from podophyllin (*Podophyllum peltatum*) For the glycosidation of the phenolic 8-hydroxyl group of **1** the Koenigs–Knorr synthesis as modified by Helferich *et al*[12] was used. The reaction of **1** with 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl chloride in acetonitrile at 20° in the presence of

*Part IV in the series Enzymically Cleavable, Specific Transport-Forms of Cancerostatic Agents.



mercury(II) cyanide gave crystalline 8-*O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl)-β-peltatin-A (**2**) in 70% yield, m p 112–115° Owing to neighboring-group participation by the benzoyl group at O-2 of the 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl chloride, the α-glycoside **2** is formed stereoselectively Mass spectrometry of **2** revealed an M^{+} peak of 858 2506 (calc . 858 2524) In addition, two fragments at *m/e* 414 1311 and 445 1262 were found, resulting from the cleavage of the glycosidic bond (see Experimental section). By using the general method of Kuhn and von Wartburg[14] for deacetylation of glycoside acetates but with increased reaction time, compound **2** was debenzoylated. No epimerization took place at C-3 of the aglycon during the debenzoylation of **2** as the enzymically cleaved aglycon gives R_F values on t l c (chloroform) identical with the starting material, whereas the epimerization product β-peltatin-B (**5**), obtained from **1** by a known procedure[13], shows a somewhat lower R_F value. The enzymically cleaved aglycon is readily soluble in chloroform, whereas **5** was reported to be less than 1% soluble in this solvent[13]. Finally, compound **3** in combination with α-L-arabinofuranosidase exerts strong cytostatic effects on tumors, as will be reported in a separate paper, whereas compound **5** has been reported to be of low cytostatic effectiveness[13].

The furanoid structure of **3** is evident from its n m r. spectrum in Me_2SO-d_6 The signals of the hydroxyl protons are well resolved HO-5 gives a triplet at δ 4 97, and HO-3 and HO-2 give doublets at δ 5 35 and 5 55 Essentially identical signals were observed, for instance, with 2-naphthyl α-L-arabinofuranoside[15]. These three signals disappear on addition of deuterium oxide Mass spectrometry showed the M^{+} peak at 546 1725 (calc for $C_{27}H_{30}O_{12}$ 546 1738), together with two fragments arising from cleavage of the glycosidic bond The α-L-glycosidic linkage was confirmed by the observed enzymic splitting by α-L-arabinofuranosidase (*Aspergillus niger* K1) to give **1** and L-arabinose The pH-dependence of the enzymic cleavage is extremely favorable for our proposed strategy, at pH 7 4 no cleavage was found during 60 min, whereas at pH 6 1 cleavage was already observed after 5 min. Increased cleavage occurred at pH 4 15

Compared with **1**, compound **3** is essentially non-toxic This is evident from comparison of the hematotoxic action of both compounds on animals. Subcutaneous injection of compound **3** on three successive days (total dose, 3 15 mg) did not cause

an abnormal leukocyte count, whereas the application of extremely small amounts of **1** (overall dose 0 24 mg) caused a considerable decrease in the leukocyte counts (Table I). Similar results were obtained in experiments using [^{3}H]thymidine to study the influence on DNA synthesis One single application of 0 3 mg of compound 3 caused no significant decrease of [^{3}H]thymidine incorporation In contrast, 0 2 mg of compound **1** caused an unusually strong inhibition of DNA synthesis, especially in the tumor and in the spleen (Table II) Further details of experiments on enzymic toxification of compound 3 in animals, and of the resulting selective gain in activity, will be reported in a separate paper.

TABLE I

LEUKOCYTE COUNTS OF MICE BEARING ASCITES SARCOMA MV 276A[20] FOLLOWING SUBCUTANEOUS INJECTION OF β-PELTATIN-A AND 8-*O*-(α-L-ARABINOFURANOSYL)-β-PELTATIN-A (3), RESPECTIVELY

Day	*β-Peltatin-A*		*Compound* 3		*Untreated leukocytes*
	Dose	*Leukocytes*	*Dose*	*Leukocytes*	
1	3 × 40 μg[a]	11,900	3 × 500 μg	8,200	
2	3 × 20 μg	2,500	3 × 400 μg	9,600	
3	3 × 20 μg	1,000	3 × 150 μg	11,200	10,000 ±2,000

[a]The daily injections were given at intervals of 2 5 h

TABLE II

INCORPORATION OF [^{3}H]THYMIDINE INTO DNA OF TRANSPLANTED MAMMARY CARCINOMA, INTESTINE, AND SPLEEN OF THE CBA MOUSE FOLLOWING A SINGLE SUBCUTANEOUS ADMINISTRATION OF 8-*O*-(α-L-ARABINOFURANOSYL)-β-PELTATIN-A (3) (0 3 mg) AND β-PELTATIN-A (0 2 mg)

	Untreated	*β-Peltatin-A*	*Compound* 3
Tumor	11,212 ±603[a]	135 ±20	9,138 ±1,888
Intestine	12,065 ±638	3,578 ±170	13,277 ±1,916
Spleen	7,136 ±486	419 ±51	5,011 ±751

[a]Values as counts/min/mg DNA

EXPERIMENTAL

General methods — Melting points were determined on a Kofler micro-hot-stage, and are uncorrected Optical rotations were measured with a VEB Carl Zeiss (Jena) polarimeter T l c was performed on 0 25-mm plates of Silica Gel F_{254} (Merck), and the spots were detected under a u v lamp. Column chromatography was effected on columns of Silica Gel H (type 60, Merck) as reported by Stahl N.m r spectra were recorded on a KRH-100 spectrometer (DAW AWF Berlin-Adlershof) at 100 MHz Chemical shifts are given in p p m. with tetramethylsilane

as internal standard ($\delta = 0.00$) Mass spectrometry was carried out on an MS-902S mass spectrometer (AEI, Manchester) at an ionizing potential of 70 eV and an accelerating potential of 8 kV The elemental composition of the fragments was obtained by high resolution (resolution 10000) Radioactive samples were counted on a Tri-Carb scintillation counter (Mark II, Nuclear Chicago)

Isolation of β-peltatin-A (**1**) — Podophyllin (55 g, from *Podophyllum peltatum* L , Merck) was added to chloroform (300 ml) The suspension was filtered and the insoluble residue was washed three times with chloroform The filtrate was concentrated *in vacuo* to 25 ml, and the solution chromatographed on silica gel (740 g) with chloroform as eluant Compound **1** (3 9 g) was obtained, giving a single spot on t.l c (R_F 0 62, 9 1 chloroform–methanol) After recrystallization from benzene and then from abs ethanol, the physical constants of **1** accorded with those reported in the literature[10,13,16,17]

8-O-(2,3,5-Tri-O-benzoyl-α-L-arabinofuranosyl)-β-peltatin-A (**2**) — A solution of dried **1** (1 5 g, 3 61 mmoles), 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl chloride[18,19] (3 12 g, 6 5 mmoles), and dry mercury(II) cyanide (1 65 g, 6 5 mmoles) in freshly distilled, dry acetonitrile (32 ml) was stirred magnetically under anhydrous conditions for ~60 h at 18–22°. After this time, compound **2** appeared as the main spot on t l c (R_F 0 58, 19 1 chloroform–acetone) The solvent was evaporated off *in vacuo* (60°) and the residue was extracted with chloroform (30 ml). Filtration (Celite) gave a clear solution that was washed with M sodium bromide (4 × 20 ml) and water (3 × 40 ml), and dried (sodium sulfate) The solution was evaporated and the product chromatographed on silica gel (720 g, column 4 × 107 cm) with 19 1 chloroform–acetone to give analytically pure **2** (2 09 g, 70%), which was recrystallized from methanol as colorless crystals, m p 112–115°, $[\alpha]_D^{25}$ −84 9° (*c* 5 0, chloroform), mass spectrum· *m/e* 858 2506 (M^+), 445 1262 ($M^+ - C_{22}H_{21}O_8$), 414 1311 ($M^+ - C_{26}H_{21}O_7 + 1H$) For elemental analysis the substance was dried *in vacuo* for 6 h at 100°.

Anal Calc. for $C_{48}H_{42}O_{15}$. C, 67 13, H, 4 93 Found· C, 66 95; H, 5 14

8-O-(α-L-Arabinofuranosyl)-β-peltatin-A (**3**) — A solution of **2** (450 mg, 0 53 mmoles) and anhydrous zinc acetate (213 mg, 1.17 mmoles) in a mixture of dry methanol (10 ml) and dry benzene (2 ml) was heated at reflux temperature under anhydrous conditions for ~25 h and the reaction was monitored by t l c (15 4 1 chloroform–methanol–water,) The debenzoylated product then appeared as a main spot (R_F 0 62) The solution was evaporated *in vacuo*, and the residue dissolved in 30 ml of 4·1 chloroform–butanol The solution was washed with water (30 ml) and the two phases were filtered The organic phase was separated from the filtrate, again washed with water (30 ml) and dried (sodium sulfate) The solution was evaporated and purified by column chromatography on a column (2 7 × 27 cm) of silica gel (70 g) with 120 12 1 chloroform–methanol–water as eluant Fractions showing a component having R_F 0 62 were pooled and evaporated to dryness, and the residue was dissolved in dry dichloromethane Evaporation of the clear solution gave amorphous **3** (148 mg, 52%), m p 110–116°, $[\alpha]_D^{25}$ −166.1° (*c* 5 0, chloroform); n m r. data (Me_2SO-d_6) δ 4.79 (t, HO-5), 5 35 (d, HO-3), 5 55 (d, HO-2) (these three signals disappeared on

addition of deuterium oxide); mass spectrum *m/e* 546 1725 (M^+), 414 1307 ($M^+ - C_5H_9O_4 + 1H$), 133.0482 ($M^+ - C_{22}H_{21}O_8$) Attempts to crystallize this product were unsuccessful Compound 3 in the Liebermann reaction (1 6 mg 3+1 6 ml acetic anhydride +2 drops of concentrated sulfuric acid) gave initially a deep blue, and then a violet color The same colours appeared with 8-*O*-(β-D-glucopyranosyl)-β-peltatin-A[11]. For elementaly analysis, a sample was dried *in vacuo* for 6 h at 100°.

Anal Calc. for $C_{27}H_{30}O_{12}$. C, 59 34, H, 5 53 Found C, 59 21, H, 5 61.

A more-slowly moving, minor component (R_F 0 53) was eluted from the column and is under investigation

pH dependence of the enzymic cleavage of compound 3. — Samples containing 0 5 mg of substrate and 0 013 units of α-L-arabinofuranosidase (*Aspergillus niger* K1) in 0 15 ml of sodium phosphate buffer (pH 7 4, 6 1, and 4 15) were incubated for 60 min at 37° At intervals, the products were separated by t l c (15 4 1 chloroform–methanol–water) The spots were detected by spraying with 20% sulfuric acid and heating for 15 min at 110°

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REFERENCES

1 A. Graffi, *Arch Geschwulstforsch*, 43 (1974) 301–309
2 K. Schwabe, H Dorn, B Tschiersch, and A Graffi, *J Prakt Chem*, 316 (1974) 429–433
3 K. Schwabe and A Graffi, *Pharmazie*, 30 (1975) 70–74
4 K Schwabe, A Graffi, J Redslob, W Hulsmann, and G Butschak, *Pharmazie*, 31 (1976) in press
5 D Arndt and A Graffi, *J Prakt Chem*, 317 (1975) 752–756
6 B Tschiersch and K Schwabe, unpublished results
7 A Kaji and K Tagawa, *Biochim Biophys Acta*, 207 (1970) 456–464
8 J Leiter, V Downing, J L Hartwell, and M J Shear, *J Nat Cancer Inst*, 10 (1950) 1273–1293
9 J L Hartwell, A W Schrecker, and J Leiter, *Proc Amer. Assoc Cancer Res*, 1 (1954) 19
10 H Emmenegger, H Stahelin, J Rutschmann, J Renz, and A von Wartburg, *Arzneimittel-Forsch*, 11 (1961) 327–333
11 A von Wartburg, E Angliker, and J Renz, *Helv Chim Acta*, 40 (1957) 1331–1357
12 B Helferich and K Weiss, *Chem Ber*, 89 (1956) 314–321; B Helferich and J Zirner, *ibid*, 95 (1962) 2604–2611, B Helferich and W Ost, *ibid*, 95 (1962) 2612–2615
13 J L Hartwell and W E Detty, *J Am Chem Soc*, 72 (1950) 246–253
14 M Kuhn and A von Wartburg, *Helv Chim Acta*, 51 (1968) 163–168
15 A H Fielding and L Hough, *Carbohydr Res*, 20 (1971) 416–417
16 J Bartek, H Ptesilova, V. Masinová and F Santavý, *Chem Listy*, 49 (1955) 1550–1560, *Coll Czech Chem Commun*, 21 (1956) 392–404
17 C Jorgensen and H Kofod, *Acta Chem Scand*, 8 (1954) 941–953
18 A K. Bhattacharya, R K Ness, and H G Fletcher, Jr, *J Org Chem*, 28 (1963) 428–435
19 G Wagner and D Gobel, *Pharmazie*, 28 (1973) 184–189
20 W Arnold, *Arch Geschwulstforsch*, in press