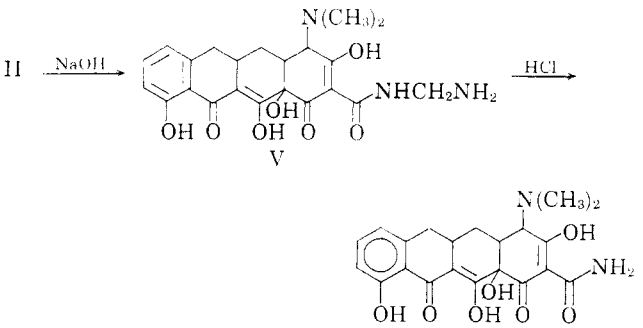
TABLE I

Derivative of 6-deoxytetracycline	R_f[a]	Partition chromatography data[b]	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
2-Carboxamido-N-phthalimidomethyl-6-demethyl-	0.83	C–D–W (5:5:1) HBV[c] 2.0–3.0	$C_{30}H_{27}N_3O_9 \cdot 0.5C_4H_8O_2$[d]	62.23	5.06	6.80	62.51	5.27	6.42
2-Carboxamido-N-maleimidomethyl-6-demethyl-	0.69	H–EA–M–W (40:60:17:6) HBV 2.7–3.6	$C_{26}H_{25}N_3O_9 \cdot 0.5H_2O$	58.64	4.92	7.89	58.30	5.06	7.52
2-Carboxamido-N-trifluoroacetamidomethyl-6-demethyl-	0.88	H–EA–ME–W (35:65:17:6) HBV 2.0–3.0	$C_{24}H_{24}F_3N_3O_8$[e]			7.79			7.95

[a] 1-Butanol–phosphate buffer, pH 2.0. [b] C = cyclohexane, D = dioxane, W = water, H = heptane, EA = ethyl acetate, M = methanol, ME = methoxyethanol. [c] HBV = hold-back volume (column solvent retention). [d] Solvent confirmed by nmr. [e] *Anal.* Calcd: F, 10.57. Found: F, 10.90.

Hydrolysis was accomplished in the case of the phthalimidomethyl derivative (III) by simple reflux in methanol using *n*-butylamine as the base.[13] Even more conveniently the trifluoroacetamidomethyl derivative II was easily decomposed by reaction with aqueous base at room temperature similar to the hydrolysis of trifluoroacetyl-protected peptides.[14] In the case of II it was also necessary to heat with dilute acid for a short time to complete the reaction, presumably to hydrolyze the intermediate aminomethyl derivative V which could be detected by paper chromatography but was not isolated. The compounds



exhibited no significant biological activity. For tests used see paper IX.[15]

Experimental Section

Descending paper chromatography was carried out on Whatman No. 1 paper buffered with 0.2 *M* pH 2 phosphate buffer, and run in a system 1-butanol–phosphate buffer pH 2.0 (2:1). Analyses were prepared by Mr. L. Brancone and staff. Liquid–liquid partition chromatography[16] was carried out on neutral (acid-washed) diatomaceous earth (Celite).

2-Carboxamido-N-phthalimidomethyl-6-demethyl-6-deoxytetracycline.—2-Decarboxamido-2-cyano-6-demethyl-6-deoxytetracycline[2c] (400 mg, 1.0 mmole) was dissolved in 6 ml of concentrated H_2SO_4 at room temperature after which 196 mg (1.1 mmoles) of N-hydroxymethylphthalimide[6] was added with stirring. The solution was stirred at room temperature for 25 min then poured slowly into 200 ml of dry ether with stirring. The precipitated solid was filtered off, washed with ether, and dried. The neutral form was prepared by slurrying the salt in 16 ml of water and adjusting the pH to a constant reading of 5.0 by the addition of 2 *N* aqueous NaOH. The solid was filtered off, washed with water, and dried, 510 mg. The crude product was purified by liquid–liquid partition column chromatography on neutral (acid-washed) diatomaceous earth as indicated in Table I, 240 mg.

In a similar manner, N-hydroxymethylmaleimide[6] and N-hydroxymethyltrifluoroacetamide[9] were treated with the nitrile.

Conversion of 2-Carboxamido-N-phthalimidomethyl-6-demethyl-6-deoxytetracycline to 6-Demethyl-6-deoxytetracycline.—A solution of 58 mg (0.1 mmole) of 2-carboxamido-N-phthalimidomethyl-6-demethyl-6-deoxytetracycline in 25 ml of dry methanol and 0.15 ml of *n*-butylamine was refluxed for 5 hr. The solution was evaporated to dryness and triturated well with dry ether and the solid material was filtered off and dried, 36 mg. Paper chromatography showed no starting material and a new spot corresponding to 6-demethyl-6-deoxytetracycline appeared at R_f 0.72. Turbidometric assay of the crude di-*n*-butylamine salt was 332 μg/ml (tetracycline = 1000).

Conversion of 2-Carboxamido-N-trifluoroacetamidomethyl-6-demethyl-6-deoxytetracycline to 6-Demethyl-6-deoxytetracycline.—A solution of 14 mg (0.025 mmole) of 2-carboxamido-N-trifluoroacetamidomethyl-6-demethyl-6-deoxytetracycline in 0.75 ml (0.075 mmole) of 0.1 *N* aqueous NaOH was allowed to stand at room temperature for 30 min, after which, 4.25 ml of 0.1 *N* methanolic HCl was added, and the resultant solution refluxed for 1 hr. The solution was evaporated to dryness, after which, the residue was dissolved in 0.5 ml of the lower phase of a solvent mixture heptane–ethyl acetate–methoxyethanol–water (60:40:15:4) and brought to pH 5 with solid sodium acetate. This solution was mixed with 1 g of diatomaceous earth and packed on a 10-g diatomaceous earth column moistened with 5 ml of lower phase, and the product was obtained by developing with upper phase; the product, 1.5 mg, being obtained in the third hold-back volume. The product was identified by ultraviolet spectrum and paper chromatography. No attempt was made to establish optimum conditions in the reaction or product isolation.

(13) L. Goldman and J. W. Marsico, *J. Med. Chem.*, **6**, 413 (1963); F. S. Spring and J. C. Woods, *Nature*, **158**, 754 (1946).

(14) F. Weygand and E. Esendes, *Angew. Chem.*, **64**, 136 (1952), and later references.

(15) M. J. Martell, Jr., A. S. Ross, and J. H. Boothe, *J. Med. Chem.*, **10**, 359 (1967).

(16) 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).

1,3,2-Diazaphosphorine 2-Oxides. IV.[1] A New Method for the Preparation of 2-(N-Arylamino)- and 2-(N-Alkylamino)-1,3,2-diazaphosphorine 2-Oxides[2]

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Received November 5, 1966

Previous articles have been concerned with the synthesis and antitumor activity of compounds of type III.[3] These were prepared by allowing a diamine, N,N′-bis(*para*-substituted benzyl)-1,3-diaminopropane I, to react with an N-arylphosphoramidic dichloride II (Chart I).

Since the synthesis of the N-arylphosphoramidic dichlorides II involves the direct addition of $POCl_3$

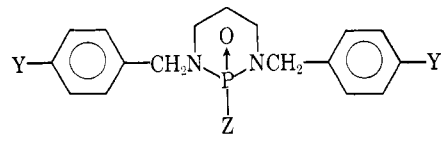
(1) Part III: J. H. Billman, J. L. Meisenheimer, and R. F. May, *J. Med. Chem.*, **9**, 772 (1966).

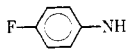
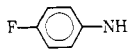
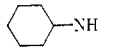
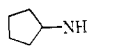
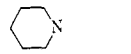
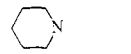
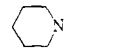
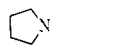
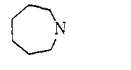
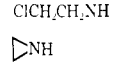
(2) This investigation was supported by Public Health Service Grant CA-06448-03 from the National Institutes of Health, Public Health Service.

(3) J. H. Billman and J. L. Meisenheimer, *J. Med. Chem.*, **8**, 264 (1965).

TABLE I

1,3-BIS(ARALKYL)-2-(N-ARYL- OR ALKYLAMINO)-1,3,2-DIAZAPHOSPHORINE 2-OXIDES



Z	Y	Yield, % (pure)	Procedure	Mp, °C[a] (cor)	Formula	Nitrogen, % Calcd	Nitrogen, % Found
	Cl	50.7	B	141–142	$C_{23}H_{23}Cl_2FN_3OP$	8.78	8.73
	$N(CH_3)_2$	34.0	B	178–179	$C_{27}H_{35}FN_5OP$	14.11	14.01
	Cl	55.0	A	120–122	$C_{23}H_{30}Cl_2N_3OP$	9.05	9.25
$(CH_3CH_2)_2N$	OCH_3	34.4	A	89–91	$C_{25}H_{39}N_3O_3P$	9.16	8.84
	Cl	75.0	A	119–121	$C_{22}H_{27}Cl_2N_3OP$	9.34	9.43
	Cl	80.5	A	111–113	$C_{22}H_{25}Cl_2N_3OP$	9.30	9.46
	OCH_3	89.0	A	78–80[b]	$C_{24}H_{34}N_3O_3P$	9.50	9.36
	$N(CH_3)_2$	71.2	A	117–119	$C_{26}H_{40}N_5OP$	14.99	15.03
	Cl	60.7	A	129–130	$C_{21}H_{26}Cl_2N_3OP$	9.59	9.65
	Cl	59.0	B	72–74	$C_{19}H_{22}Cl_2N_3OP$	10.24	10.14
	Cl	72.6	B	103–105	$C_{23}H_{30}Cl_2N_3OP$	9.03	9.36
$ClCH_2CH_2NH$	Cl	72.5	A	120–121	$C_{19}H_{23}Cl_3N_3OP$	9.41	9.28
	$N(CH_3)_2$	55.2	B	121–122	$C_{24}H_{36}N_5OP$	15.80	15.57

[a] All samples were recrystallized from acetonitrile unless designated. [b] Recrystallized from ethyl acetate.

CHART I

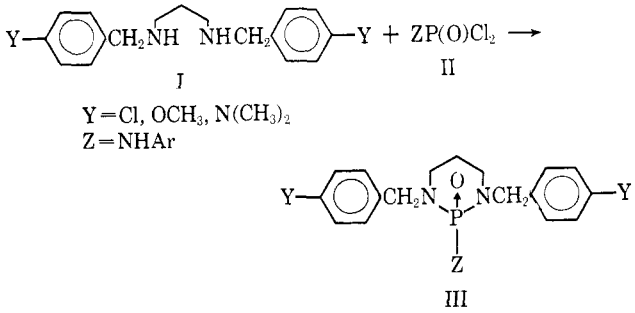
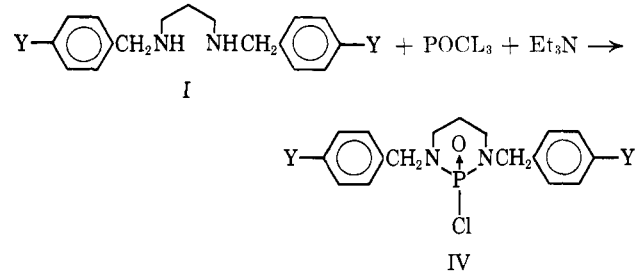


CHART II



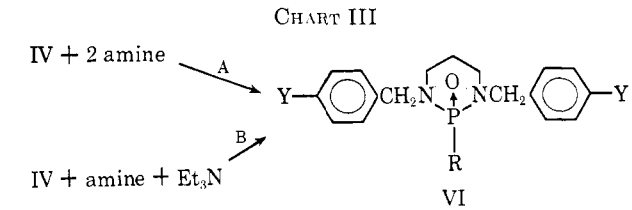
to the amine hydrochloride,[4] one is limited to the use of amines containing functional groups that are not altered by refluxing $POCl_3$. In order to overcome the limitations leading to compounds of type III, a new series of intermediates of type IV was prepared.[1] It has been demonstrated in this laboratory that 1,3-bis-(aralkyl)-2-chloro-1,3,2-diazaphosphorine 2-oxides (IV) can be readily prepared from secondary amines of type I and $POCl_3$ according to Chart II.[1]

In order to check the reactivity of the 1,3-bis-(aralkyl)-2-chloro-1,3,2-diazaphosphorine 2-oxides (IV) and to compare the effectiveness of this approach with that shown in Chart I, a series of amines were treated with the diazaphosphorine 2-oxides IV. A comparison of yields indicates that this new approach is equal in every respect to the standard method and at the same time has the added advantage of allowing one to prepare more complex molecules.

In antitumor screening, change of the electronic nature of Y had little or no effect on antineoplastic properties.

The compounds of type VI (Chart III) are summarized in Table I.

CHART III



Biological Results.—The 13 compounds summarized in Table I were submitted to the Cancer Chemotherapy National Service Center for antitumor screening.

(4) A. Michaelis and G. Schulze, *Ber.*, **26**, 2938 (1893).

They were tested against Walker carcinosarcoma 256, Sarcoma 180, Friend virus leukemia, and Lewis lung carcinoma. Available biological data on all of these compounds suggest that they are nontoxic but inactive in the tested systems.

Experimental Section

All melting points were taken on a Thomas–Hoover melting point apparatus and are corrected. The analyses were performed by Midwest Microlaboratories, Inc., Indianapolis, Ind. All amines were purified either by distillation or crystallization from appropriate solvents.

1,3-Bis(aralkyl)-2-(N-aryl- or alkylamino)-1,3,2-diazaphosphorine 2-Oxides (Table I).—The synthesis of these compounds is illustrated well by two basic procedures which shall be referred to as procedure A and procedure B.

Procedure A is typified by the synthesis of 1,3-bis(*p*-chlorobenzyl)-2-(N-piperidyl)-1,3,2-diazaphosphorine 2-oxide. 1,3-Bis-(*p*-chlorobenzyl)-2-chloro-1,3,2-diazaphosphorine 2-oxide, 5.0 g (0.012 mole), was dissolved in 75 ml of benzene. Piperidine, 2.1 g (0.025 mole), was added dropwise to the refluxing solution over a 20-min period and a precipitate formed at once. After the complete addition of the amine, the reaction mixture was refluxed for 15 min and allowed to cool. The piperidine hydrochloride, 1.38 g (0.011 mole), was collected by filtration. The benzene from the filtrate was removed completely under reduced pressure and the resulting oil was dissolved in a minimum of acetonitrile. After setting for several days at 0°, 4.5 g of white crystals were collected by filtration (mp 110–112°). On recrystallization of a small sample, a pure solid, melting at 111–113° was obtained. The crude yield was 80.5%.

Anal. Calcd for $C_{22}H_{25}Cl_2N_3OP$: N, 9.30. Found: N, 9.46.

Procedure B is illustrated by the preparation of 1,3-bis(*p*-chlorobenzyl)-2-(N-aziridyl)-1,3,2-diazaphosphorine 2-oxide. Aziridine (0.54 g, 0.012 mole), and 2 ml of triethylamine in benzene were added dropwise, over a 15-min period, to a benzene solution of 5.0 g (0.012 mole) of the 1,3-bis(*p*-chlorobenzyl)-2-chloro-1,3,2-diazaphosphorine 2-oxide. A precipitate of triethylamine hydrochloride formed at once and refluxing was continued for 15 min after complete addition of the amine solution. The solid hydrochloride was removed by filtration and the benzene was completely removed under reduced pressure to give 4.0 g (79% yield) of a white solid. The product was recrystallized from acetonitrile to give 3.0 g of pure product in 59% yield, mp 72–74°.

Anal. Calcd for $C_{19}H_{22}Cl_2N_3OP$: N, 10.24. Found: N, 10.14.

Scopoletin, an Antispasmodic Component of *Viburnum opulus* and *prunifolium*

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The genus *Viburnum* contains several notable species, particularly *opulus* and *prunifolium*, which were used in American Indian therapeutics.[1] Their utilization has persisted into recent times[2] but their efficacy has been questioned frequently. We have verified both to have a reasonably high order of antispasmodic activity and that this is due to several compounds.[3]

Until recently[4-6] chemical explorations of *V. opulus* and *V. prunifolium* have been limited and largely inconclusive, especially with respect to biologically active components. Our work on the antispasmodic and cytotoxic components of these plants has required large quantities of extracts and in their fractionation we have obtained crystalline scopoletin from both, a finding contradictory to earlier work.[5]

A determination of whether scopoletin had antispasmodic properties was made using an *in vitro*, estrone-primed and barium chloride stimulated rat uterus preparation.[3] The compound was found to be reasonably active and the average bath concentration required to produce a 50% decrease in contraction amplitude, ID_{50}, of single uterine horns was 0.09 mg/ml. A qualitatively similar effect was noted in whole animal experiments utilizing oxytocin- and ergonovine-promoted uterine contractions. The smooth muscle relaxant property of scopoletin is an unusual effect for coumarin compounds, but not surprising in view of the number which show activity as nonspecific vasodilators.[7]

Experimental Section

V. prunifolium.—Root bark (12 kg) was extracted with distilled water in an Eppenbach stirrer. The aqueous filtrate was extracted with CH_2Cl_2 to yield 9.5 g (0.08%) of fluorescent red oil. The crude product was put on a 3 × 7 cm column of 50 g of Woelm activity grade I neutral alumina and eluted with 600 ml of redistilled $CHCl_3$. Evaporation of the solvent gave 5.3 g (0.04%) of bright yellow fluorescent oil. It was dissolved in 50 ml of cold methanol, cooled to 5°, and centrifuged for 15 min to precipitate the waxes. The suspension was filtered to give 4.5 g (0.04%) of oil. It was dissolved in 20 ml of $CHCl_3$, concentrated to 7 ml, and cooled to 5° for 24 hr to yield slightly yellow needles, mp 195–205°. The product was washed with cold $CHCl_3$ and crystallized from 5% methanol–$CHCl_3$ to yield 50 mg (4×10^{-4}%) of scopoletin, mp 210° (lit.[8] mp 204–205°), mmp 210°.

V. opulus.—Root bark (22.5 kg) was extracted to yield 126 g (0.56%) of oil. When chromatographed as above 103 g (0.46%) of brown oil was obtained. The crude product was mixed with sand and extracted with petroleum ether (bp 30–50°, alumina purified) for 280 hr. The extract was concentrated to 250 ml, diluted to 500 ml with ethyl ether, cooled to 10°, and extracted with cold 1 *N* NaOH. The extract was immediately acidified with 6 *N* HCl and back extracted with ethyl ether to yield 38.8 g (0.17%) of light brown oil which was refrigerated to precipitate 0.17 g (7.5 × 10^{-4}%) of light yellow scopoletin. On crystallization from methanol–water the melting point was 204.5–205.5°.

Thin Layer Chromatography of Scopoletin.—Purity and completeness of separation were determined using Merck silica gel G and H cast into 20 cm × 17 mil films from 28.5% distilled water slurries. The films were methanol washed by upward development, stored at 100° for 12 hr, desiccated, and then activated for 0.5 hr at 100° just prior to use. The solvent system used was water-saturated ethyl ether: R_f (plate G) 0.28, (plate H) 0.44.

Bioassay of Scopoletin.—Potency was determined using a micro version of the reported method.[3] The total bath volume was 10 ml and regular uterine contractions were induced with just sufficient 1% $BaCl_2$ to produce maximum effect. Scopoletin was dissolved in propylene glycol to give 5 mg/ml and cumulative dose–response experiments were performed. Bath concentrations of 0.025, 0.050, 0.100, and 0.150 mg/ml were used. For each con-

(1) (a) H. W. Youngken, *J. Am. Pharm. Assoc.*, **19**, 680 (1930); (b) J. C. Munch, *Pharm. Arch.*, **11**, 33 (1940); (c) C. H. Costello and E. V. Lynn, *J. Am. Pharm. Assoc.*, **32**, 20 (1943); (d) R. A. Woodbury, *Drug Std.*, **19**, 143(1951).

(2) "The Dispensatory of the United States of America," A. Osal and G. E. Farrar, Ed., 25th ed, J. B. Lippincott Co., Philadelphia, Pa., 1960.

(3) C. H. Jarboe, C. M. Schmidt, J. A. Nicholson, and K. A. Zirvi, *Nature*, **212**, 837 (1966).

(4) J. M. Bobbitt and K. V. Rao, *J. Pharm Sci.*, **54**, 924 (1965).

(5) L. Hoerhammer, H. Wagner, and H. Reinhart, *Naturwissenschaften*, **52**, 161 (1965).

(6) L. Hoerhammer, H. Wagner, and H. Reinhardt, *Deut. Apotheker-Ztg.*, **105**, 1371 (1965).

(7) T. O. Soine, *J. Pharm. Sci.*, **53**, 231 (1964).

(8) W. Karrer, "Konstitution und Vorkommen der Organischen Pflanzenstoffe," Birkhäuser Verlag, Basel, 1958, p 537.