TABLE IV

EFFECT OF CANDIDATE CHEMOSTERILANTS IN THE FOOD OF ADULT HOUSEFLIES IN SCREENING TESTS (BOTH SEXES MAINTAINED ON TREATED DIET FROM EMERGENCE TO OVIPOSITION)

		Treated diet				Untreated diet	
Compd	Type of food	Concn, %	Mortality, parent generation, %	Egg hatch, %	Pupae, no.	Egg hatch, %	Pupae, no.
1	Sugar	1.0	11	84	84	86	86
	Fly food	1.0	0	87	87	88	88
2	Sugar	1.0	11	56	56	86	86
	Fly food	1.0	0	70	70	88	88
3	Sugar	1.0	0	91	91	86	86
	Fly food	1.0	0	87	87	88	88
4	Sugar	1.0	21	79	79	86	86
	Fly food	1.0	0	90	90	88	88
5	Sugar	1.0	11	79	79	86	86
	Fly food	1.0	0	71	71	88	88

Discussion

Table IV summarizes the results of the chemosterilant test in houseflies. None of these compounds were significantly active in contrast to the corresponding aziridine analogs previously studied.[3]

Table V summarizes the results of the evaluation of these compounds in the male mouse. None of the methanesulfonate derivatives had activity comparable to that of the corresponding aziridine analogs. However, the C_{16} derivative, **4**, does seem to have a delayed effect as evidenced by reduction of litter sizes in weeks 9–14. This effect is similar to that observed by Jackson in the case of busulfan and isopropyl methanesulfonate which affected sterility only after several weeks.[5]

Acknowledgment. This work was supported by Stanford Research Institute's Research and Development Program. We wish to thank Mr. Tong for the mice studies and Dr. Weidhaas and J. Roos for the housefly studies.

TABLE V

EFFECT ON REPRODUCTION IN THE MALE SWISS-WEBSTER MOUSE

		Weeks														
		Litter size														
Compd	Dose, oral, mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	No. of sires
2	5 × 40	10	10	9	10	11	13	5	7	2	7	11	3	14	4	2
3	5 × 40	5	6	4	12	12	7	9	5	5	8	6	5	9	7	2
4	5 × 40	10	12	12	10	11	9	5	12	4	9	9	9	3	4	2
Control	Saline	1	2	7	2	9	10	9	7	3	8	4	4	10	8	3

SCHEME I

$$H_2N(CH_2)_nNH_2 \xrightarrow{ClCOCH_2OCOCH_3} (CH_3OCOCH_2CONH)_2(CH_2)_n \xrightarrow[MeOH]{Na} (HOCH_2CONH)_2(CH_2)_n \xrightarrow{ClSO_2Me} (CH_3O_2SOCH_2CONH)_2(CH_2)_n$$

perature for 2 hr, and then at reflux for another 2 hr. The solvent was evaporated *in vacuo* and the residue was washed with water giving the crude product. Recrystallization from the solvents indicated (Table I) gave analytical samples.

N,N'-Bis(hydroxyacetyl)-α,ω-alkylenediamine.—The *N,N'*-bis(carbomethoxyacetyl)-α,ω-alkylenediamine (5 mmol) was dissolved in 60 ml of MeOH in which an analytical amount of Na has been dissolved. The reaction mixture was stirred at room temperature for 2 hr. Evaporation of the solvent *in vacuo* left a white crystalline product. Recrystallization (Table II) gave analytical samples.

N,N'-Bis(methanesulfonyloxyacetyl)-α,ω-alkylenediamine.—$MeSO_2Cl$ (4 mmol) in 2 ml of Et_2O was added slowly with cooling to the *N,N'*-bis(hydroxyacetyl)-α,ω-alkylenediamine (2 mmol) dissolved in a mixture of 2 ml of pyridine and 4 ml of Et_2O. After stirring for 2 hr at room temperature, Et_2O was evaporated and ice water was added to the residue. The white precipitate was filtered, dried, and recrystallized (AcMe–Et_2O) to give the compounds (Table III).

Biological Methods

Housefly Chemosterilant Assay.—These studies were conducted by the U. S. Department of Agriculture at its Insects Affecting Man and Animals Research Laboratory, Gainesville, Fla. The method used was essentially that previously reported.[4]

Mice Chemosterilant Assay.—These studies were conducted by H. C. Tong, Stanford Research Institute, by the method previously reported.[2]

(4) G. C. LeBreque, *J. Econ. Entomol.*, **54**, 684 (1961).

(5) R. Jackson, "Antifertility Compounds in the Male and Female," C. C. Thomas, Springfield, Ill., 1966, pp 59, 72.

Cassaine Analogs. VI.[1] Resolution of (±)-7-Deoxy-16,17,18,20-tetranorcassaic Acid and Biological Activity of Derived Esters

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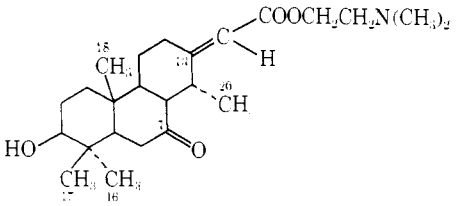
Recently we described a series of simplified analogs of the *Erythrophleum* alkaloid cassaine (**1**)[2] which were prepared in an effort to find a synthetically feasible and medicinally acceptable analog of cassaine, a cardiotonic agent. These simplified structures, exemplified by formula **2**, proved to be only 0.1 (or less) as active as the natural product. It was of immediate concern whether at least half of the loss of activity could be attributed to the racemic nature of the synthetic compounds. Cassaine is a single (levorotatory) enantiomer.

Many efforts at resolving various racemates at several stages in the synthetic sequences reported ear-

(1) Paper V, S. J. Daum and R. L. Clarke, *J. Med. Chem.*, **11**, 1069 (1968).

(2) (a) R. L. Clarke, S. J. Daum, P. E. Shaw, T. G. Brown, Jr., G. E. Groblewski, and W. V. O'Conner, *ibid.*, **10**, 582 (1967); (b) *ibid.*, **10**, 593 (1967).



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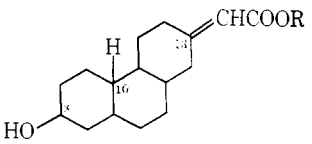


2, R = $CH_2CH_2N(CH_3)_2$
3, R = H
4, R = C_2H_5

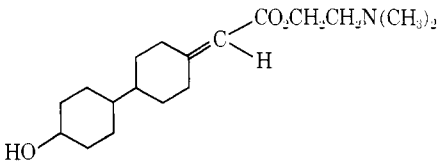
lier[2a] were unsuccessful. Gottstein and Cheney[3] have called attention to the merits of dehydroabietylamine as a resolving agent. To our considerable delight, addition of this amine to a 1:1 mixture of (±)-*trans* and (±)-*cis* acids **3** (isomeric about the *exo* double bond) resulted in preferential precipitation of the amine salt of the (−)-*trans* acid. The rotation of this salt and its high yield indicated that it was contaminated with some (−)-*cis* acid salt. However, liberation of the free acid and recrystallization afforded the desired (−)-*trans* enantiomer.

It did not prove possible to separate the (+)-*trans* acid **3** from the complex mother liquors. Yet this (+) acid was required in order to obtain both optically active basic esters for biological testing. The most likely approach at this juncture apparently lay through resolution of pure (±)-*trans* acid **3** (free of the *cis* form). Multiple recrystallization of a 1:1 *cis–trans* mixture of ethyl esters **4**[2a] enriched the *trans* isomer to 93%. Hydrolysis to acid **3** and further recrystallization then afforded a (±)-*trans* acid containing only 1.6% of the *cis* isomer.

Resolution of this (±)-*trans* acid **3** was then accomplished through its dehydroabietylamine salt, but again with only partial success. The (−)-*trans* acid was isolated without difficulty but the (+)-*trans* acid could not be obtained entirely pure. It was then discovered that the (+)-1-(1-naphthyl)ethylamine[4] salt of the (+)-*trans* acid **3** is only one-tenth as soluble in MeOH as is the diastereoisomeric (+)-amine-(−)-acid salt. Separation of the (+)-*trans* acid **3** in this manner afforded it in pure form.

In order to have the physical constants of the enantiomeric salt available to confirm melting points and rotations, the (−)-1-(1-naphthyl)ethylamine salt of (−)-*trans* acid **3** was prepared and the free (−) acid subsequently regenerated from it. The optical rotatory disperson data for these enantiomeric acids **3** are recorded in the Experimental Section.[5]

Pure (+)-*trans* and (−)-*trans* acids **3** were converted into the corresponding basic esters **2** by reaction of the acid chlorides with dimethylaminoethanol.[2a] Evaluation of the cardiotonic activity of these enantiomeric basic esters in both isolated rabbit heart atrial strips and in intact dogs according to the methods reported in the preceding paper of this series[1] showed no significant difference in their activities.[6] Perhaps this is not too surprising since there is little difference in the activities of the (±)-*cis* and the (±)-*trans* basic esters **2** (isomeric about the double bond); also little



5

difference when the rings are fused in a *trans–trans*, a *cis–trans*, or a *trans–cis* manner.[2a] Yet the activity drops significantly when the ridigity afforded by ring B is eliminated (structure **5**).[2a]

Concerning the decomposition of dehydroabietylamine salts, they should be treated with excess base and the desired acids then liberated from the aqueous phase. We found that dehydroabietylamine hydrochloride is so insoluble in H_2O that treatment of diastereoisomeric salts of dehydroabietylamine with HCl gives difficult mixtures of free acid and amine hydrochloride.

Experimental Section[7]

Separation of (±)-*trans*-Acid 3 Directly from a Mixture of (±)-*cis* and (±)-*trans* Acids.—A solution of 17.4 g (0.066 mol) of a 43:57 mixture of (±)-*cis* and (±)-*trans* acids **3**[2a] in 250 ml of hot MeOH was treated with 18.8 g (0.066 mol) of solid dehydroabietylamine.[3] The amine dissolved and immediately a crystalline precipitate formed. The mixture was cooled to 45° and the solid (16.4 g) was collected. Recrystallization from 1500 ml of absolute EtOH (boiled down to 600 ml and cooled to 5°) afforded 12.5 g of needles, mp 227–229° dec (evac tube), $[\alpha]^{25}D$ −22.6° (1% in HOAc). A second recrystallization gave 9.13 g of needles of (−)-*trans* acid **3** dehydroabietylamine salt, mp 227–228.5° dec (evac tube), $[\alpha]^{25}D$ −24.6°. *Anal.* ($C_{36}H_{55}NO_3$) C, H, N.

The (−)-*trans* acid salt (9.0 g) was suspended in 200 ml of Et_2O and 50 ml of 1 *N* HCl was added. The mixture was shaken well and filtered to separate a solid. The residue resulting from concentration of the Et_2O layer was added to this solid. The total solid was shaken with 300 ml of H_2O, 20 ml of 2 *N* NH_4OH and 100 ml of Et_2O. Acidification of the aqueous layer gave a crystalline acid which was washed well with H_2O and air dried. This acid was recrystallized from 500 ml of MeCN (concentrated to a 250-ml volume) to give 2.32 g of (−)-*trans* acid **3**, mp 217–219° (evac tube), $[\alpha]^{25}D$ −48.6° (1% in EtOH). A second recrystallization raised the melting point to 219–220° (evac tube); $[\alpha]^{25}D$ −49.1° (1% in EtOH).

Ethyl(±)-*trans*-3,4,4aα,4bβ,5,6,7,8,8aα,9,10,10aβ-Dodecahydro-7β-hydroxy-$\Delta^{2(1H),\alpha}$-phenanthreneacetate (**4**).—The crude 1:1 mixture of *cis* and *trans* isomers of this compound (41 g) prepared as described earlier[2a] was dissolved in 100 ml of cyclohexane and the solution cooled to give 16.5 g of a 3:7 mixture of *cis* and *trans* isomers. A second recrystallization from cyclohexane furnished

(3) W. J. Gottstein and L. C. Cheney, *J. Org. Chem.*, **30**, 2072 (1965).

(4) Sold as Resoline-A by the Research and Development Department, Rock Hill Laboratory, Newport, Tenn.

(5) We thank Dr. Yash P. Myer for determination of these dispersion curves.

(6) We thank Dr. G. E. Groblewski of these laboratories for this biological information.

(7) All melting points are corrected. Nmr spectral measurements were made using a Varian A-60 spectrophotometer with $(CH_3)_4Si$ as an internal indicator. Ir spectra were recorded on a Model-21 Perkin-Elmer spectrophotometer and uv spectra were measured on a Model 15 Cary spectrophotometer. Brinckmann Instruments silica gel grade PF_{254} was used in 1-mm thickness for preparative plate chromatography. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ± 0.4% of the theoretical values.

15.2 g of needles, mp 96–114°. Recrystallization of this mixture from 150 ml of Et_2O concentrated to 50 ml afforded 7.35 g of needles, mp 118–126.5°. Recrystallization from hexane three more times raised the melting point finally to 126–128.5° (5.9 g, 14%); λ_{max}^{EtOH} 223 mμ (ε 20,400); λ_{max}^{KBr} 5.84 (CO) and 6.09 μ (*exo* C=C); nmr peaks at 333 c/s (=CH), 247 Hz (OCH_2 ester). *Anal.* ($C_{18}H_{28}O_3$) C, H.

This product was shown by glpc to contain 93% of **4** and 7% of the *cis* isomer with 0.3% foreign material present. Further purification of this *trans* isomer was accomplished in the next step as described below.

(±) *trans*-3,4,4aα,4bβ,5,6,7,8,8aα,9,10,10aβ-Dodecahydro-7β-hydroxy-Δ$^{2(3H),\alpha}$-phenanthreneacetic Acid (3).—A solution of 5.7 g (0.019 mol) of the 93% pure (±) *trans* ester (**4**) in 100 ml of hot MeOH was treated with 50 ml (0.10 mol) of 2 *N* NaOH solution and the mixture was refluxed for 1 hr. The MeOH was removed by warming *in vacuo* and 200 ml of H_2O and 100 ml of Et_2O were added. This mixture was shaken thoroughly and then filtered to separate a considerable quantity of undissolved Na salt of the product. The H_2O layer from the filtrate was combined with the solid and the suspension was made strongly acidic with 2 *N* HCl. The precipitated acid was collected, air dried (5.7 g), and recrystallized twice from MeCN to afford 3.7 g (74%) of (±)-*trans* acid **3**, mp 221–224° (evac cap.). A third recrystallization of a portion of the product gave the analytical sample as fine, colorless needles of mp 225–227° (evac tube). Glpc on the Me ester derivative, prepared with CH_2N_2, showed it to contain 1.6% of the *cis* isomer. *Anal.* ($C_{16}H_{24}O_3$) C, H; neutralization equivalent: calcd, 264.4; found, 261.

Resolution of (±) *trans*-3,4,4aα,4bβ,5,6,7,8,8aα,9,10,10aβ-Dodecahydro-7β-hydroxy-Δ$^{2(3H),\alpha}$-phenanthreneacetic Acid (3).—A solution of 3.70 g (0.014 mol) of the (±) *trans* acid **3** in 50 ml of hot MeOH was treated with a solution of 4.00 g (0.014 mol) of dehydroabietylamine in 10 ml of hot MeOH. A crystalline salt precipitated immediately. The mixture was cooled and filtered to give 5.19 g of solid. The filtrate will be referred to below as the *original filtrate*.

Recrystallization of this salt twice from absolute EtOH with cooling only to room temperature produced 2.83 g of needles of the (−) *trans* acid dehydroabietylamine salt, mp 232–234° dec (evac tube). A third recrystallization gave 2.40 g of this salt which decomposed at 232.5–234.5° (evac tube); $[\alpha]^{25}D$ −19.7° (1% in HOAc). *Anal.* ($C_{36}H_{55}NO_3$) C, H, N.

The mother liquor from the recrystallization of the 5.19 g of solid was concentrated to half volume and 0.47 g of needles was obtained showing $[\alpha]^{25}D$ −3° (1% in HOAc). Concentration of the filtrate to dryness yielded 1.27 g of solid, $[\alpha]^{25}D$ +36° (1% in HOAc). This dextrorotatory residue was combined with the residue from the original filtrate and this solid was shaken with 85 ml of H_2O, 15 ml of 2 *N* NaOH, and 100 ml of Et_2O. The Et_2O layer was separated and washed with two 15-ml portions of H_2O. Acidification of the combined H_2O layer and washings gave a crystalline precipitate which was washed well with H_2O and air dried; 1.57 g, $[\alpha]^{25}D$ +40.3° (1% in EtOH). Multiple recrystallization of this enriched (−) *trans* acid **3** from MeCN failed to raise the melting point above 212–216°; $[\alpha]^{25}D$ +43.0° (1% in EtOH). Isolation of pure (−) *trans* acid from this mixture is described later in this experiment.

The (−)-*trans* acid dehydroabietylamine salt described earlier in this experiment (2.25 g) was shaken with 85 ml of H_2O, 15 ml of 2 *N* NaOH, and 100 ml of Et_2O and the layers were separated. The Et_2O layer was washed with H_2O and the combined aqueous layers were acidified with 2 *N* HCl. The precipitated (−) *trans* acid **3** was collected, washed with H_2O, and dried; 1.03 g.

A sample of this (−) *trans* acid just described (0.87 g, 3.3 mmol) was converted into its (−)-1-(1-naphthyl)ethylamine salt by dissolving in 25 ml of hot MeOH and adding a solution of 0.57 g (3.3 mmol) of the (−)-amine in 10 ml of MeOH. Concentration of the resulting solution *in vacuo* to a 5-ml volume, dilution with 35 ml of Et_2O, and filtration gave 1.31 g (91%) of (−) *trans* acid **3** (−)-1-(1-naphthyl)ethylamine salt, mp 229–231° with intumescence (evac tube). Recrystallization from 110 ml of MeOH (with concentration to 25 ml) gave 1.06 g of colorless plates and prisms, mp 231–233.5° with intumescence, $[\alpha]^{25}D$ −27.5° (1% in HOAc). *Anal.* ($C_{28}H_{37}NO_3$) C, H, N.

Treatment of 0.96 g of this (−)-acid–(−)-base salt with base in the usual manner followed by acidification of the aqueous solution gave a crystalline acid which was recrystallized from 100 ml of MeCN with concentration to 35 ml. Colorless needles of (−)-*trans* acid **3** were obtained, mp 219–220° (evac tube); RD in MeOH (*c* 0.10), 23–25°; $[\alpha]_{589}$ −49°, $[\alpha]_{500}$ −440°, $[\alpha]_{350}$ −3210°, $[\alpha]_{325}$ 0°, $[\alpha]_{306}$ +2820°, $[\alpha]_{250}$ +2090°. *Anal.* ($C_{16}H_{24}O_3$) C, H; neutralization equivalent: calcd, 264.4; found, 261.

According to the 1.57 g of enriched (+) *trans* acid described in the second paragraph of this experiment, a solution of this sample in 50 ml of warm MeOH was treated with a solution of 1.02 g of (+)-1-(1-naphthyl)ethylamine[3] in 5 ml of MeOH. Cooling to 0° and filtration afforded 1.84 g of colorless blades of the (+) *trans* acid (+)-amine salt, mp 230–231.5° (intumescence) (evac tube); $[\alpha]^{25}D$ +29.2° (1% in HOAc). The filtrate was concentrated to a residue which was triturated twice with Et_2O and then recrystallized from 25 ml of MeOH. Thus was obtained 0.22 g of salt melting at 228.5–230° (intumescence) (evac tube). The combined crops were recrystallized from 150 ml of MeOH by concentrating the solution to 60 ml and cooling to 0°. This process gave 1.54 g of blades, mp 230–231° (intumescence) (evac tube); $[\alpha]^{25}D$ +29.3°. *Anal.* ($C_{28}H_{37}NO_3$) C, H, N.

This (+) *trans* acid (+)-amine salt (1.98 g) was shaken with a mixture of 65 ml of H_2O, 10 ml of 2 *N* NaOH, and 50 ml of Et_2O and the layers were separated. The H_2O layer was acidified with 2 *N* HCl and the precipitated (+) *trans* acid **3** was washed with H_2O and dried (1.17 g). One recrystallization from MeCN gave 0.98 g of colorless needles, mp 218–220° (evac tube), and one further recrystallization raised this melting point to its maximum at 219–220° (evac tube); RD in MeOH (*c* 0.10), 23–25°; $[\alpha]_{589}$ +49°, $[\alpha]_{500}$ +785°, $[\alpha]_{350}$ +3940°, $[\alpha]_{325}$ 0°, $[\alpha]_{312}$ −1990°, $[\alpha]_{280}$ −1510°. *Anal.* ($C_{16}H_{24}O_3$) C, H; neutralization equivalent: calcd, 264.4; found, 259.

2-Dimethylaminoethyl (+) *trans*-3,4,4aα,4bβ,5,6,7,8,8aα,9,10,10aβ-Dodecahydro-7β-hydroxy-Δ$^{2(3H),\alpha}$-phenanthreneacetate (2).—A solution of 0.99 g (3.4 mmol) of the (+) *trans* acid **3** in 10 ml of MeOH was treated with 3.40 ml (3.4 mmol) of 1 *N* aqueous NaOH and the resulting solution was concentrated to a residue, by warming *in vacuo*. This residue was dissolved in 15 ml each of absolute EtOH and C_6H_6, the solution was concentrated to a residue, and the process was repeated. Then 25 ml of C_6H_6 was added and distilled with the result that a dry, colorless powder was obtained.

A stirred suspension of this Na salt in 25 ml of dry C_6H_6 and 0.20 ml of C_5H_5N was maintained at 10–15° while 5.0 ml of $(COCl)_2$ was added in 4 min. This mixture was stirred for 20 min at the same temperature and was then concentrated to a residue, at <30° *in vacuo*. The residue was suspended in 25 ml of dry C_6H_6 and treated with 6.0 ml of $Me_2NCH_2CH_2OH$ dropwise with stirring at 10–15° in 3 min. Then the mixture was boiled for 15 min with frequent stirring.

The cooled reaction mixture was diluted with 10 ml of H_2O, 10 ml of 2 *N* NH_4OH, and 50 ml of Et_2O. The layers were separated and the aqueous layer was washed once with Et_2O. These Et_2O layers were combined, washed with brine, and concentrated to a residual oil. This oil was chromatographed on six 20 × 40-cm thick-layer silica gel plates using 3:3:94 MeOH–*i*-$PrNH_2$–$CHCl_3$ for development. The principal uv-absorbing band was scraped off and eluted with freshly distilled THF. It was necessary to repeat the chromatographic process in order to remove a small quantity of less-polar impurity. Recrystallization of the 0.44 g of crystalline product by dissolving it in 25 ml of Et_2O, filtering the solution through charcoal, concentrating to 9 ml, and diluting with 20 ml of pentane gave 0.36 g of colorless needles, mp 112–114°. Recrystallization from Et_2O and pentane in the same manner gave 0.27 g (24%) of material melting at 113.5–114.5°. Recrystallization of this sample by dissolving it in 15 ml of Et_2O and concentrating the solution to 1.5 ml followed by cooling to −5° produced a different polymorphic form of the product as triangular plates. It underwent partial melting at 107–108° with resolidification and finally melted at 115–116°. When the temperature of the melting point bath was held at 102–105° for 5 min, the sample failed to show the transitional melting and simply melted at the higher temperature. It showed $[\alpha]^{25}D$ +36.5°. *Anal.* ($C_{20}H_{33}NO_3$) C, H, N.

2-Dimethylaminoethyl (+) *trans*-3,4,4aα,4bβ,5,6,7,8,8aα,9,10,10aβ-Dodecahydro-7β-hydroxy-Δ$^{2(3H),\alpha}$-phenanthreneacetate (2).—This basic ester was prepared from 1.90 g of the (+) *trans* acid **3** in a manner identical with that described for the (+) *trans* ester **2** immediately above. The chromatographed product, 0.86 g, was recrystallized twice from ether and pentane as above to give 0.54 g (22%) of colorless needles, mp 113.5–114.5°, $[\alpha]^{25}D$ +36.0°. *Anal.* ($C_{20}H_{33}NO_3$) C, H, N.