III). The assay error exceeded 5% for all samples containing 0.50 μg/ml or less of pentobarbital. The assay error was below 5% for all samples containing pentobarbital between 0.75 and 15.0 μg/ml of serum.

The method is specific for pentobarbital. Except for thiopental, it was resolved from 13 other barbiturates (Fig. 2). A similar quantitative determination for these barbiturates should be applicable.

This rapid assay can be used to conduct single oral dose pentobarbital bioavailability studies in adult and pediatric patients using only 0.1 ml of serum. This procedure also is applicable to the general screening of some other barbiturates in biological fluids.

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Separation, Identification, and Quantitation of Anthralin and Its Decomposition Products

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Abstract □ Anthralin and its decomposition products were separated by both column chromatographic and TLC techniques. Two decomposition products were characterized by TLC, melting-point data, and UV and IR spectroscopy. Pure anthralin and its decomposition products also were determined quantitatively.

Keyphrases □ Anthralin—column chromatographic and TLC analysis and separation from decomposition products □ Chromatography, column—analysis and separation of anthralin from decomposition products □ TLC—analysis and separation of anthralin from decomposition products □ Decomposition products, anthralin—column chromatographic and TLC analysis and separation □ Antipsoriatic agents—anthralin, column chromatographic and TLC analysis and separation from decomposition products

The first use of anthralin (1,8,9-anthracenetriol) for topical therapy of psoriasis was noted in a report on chrysarobin, a related compound, in 1878 (1). Psoriasis is a skin disease characterized in part by epidermal hyperplasia and, therefore, should be controllable with antimetabolic drugs. It has been assumed that anthralin acts by blocking cell division (2). Although local treatment of psoriasis vulgaris with corticosteroid creams under plastic occlusion is accepted widely, psoriasis is still treated with anthralin pastes (USP XIX) (3–6).

In treating psoriatic lesions with commercial anthralin, two side effects were documented: staining of skin and clothing and irritation of surrounding normal skin. Anthralin is relatively unstable in the presence of air, light, and heat (7). Since commercial anthralin samples contain degradation products, it was not clear what relationship existed between anthralin and its degradation products with regard to therapeutic effectiveness and side effects. It was reported (2, 8, 9) that any therapeutic effect in psoriasis results from anthralin and not its decomposition products. These findings supported that of Comaish *et al.* (10), who noted that discolored anthralin pastes (possibly containing large amounts of the quinone) are ineffective in treating psoriasis.

Table I—R_f Values for Anthralin and Its Decomposition Products

Solvent System	Plates	R_f (×100) Anthralin	Danthron	Dianthrone
Benzene–cyclohexane–methanol (15:15:0.25)	Silica	42	35	21
Benzene–cyclohexane–acetic acid (15:15:0.5)	Silica	31	20	11
Benzene–cyclohexane–methanol (15:15:0.25)	Compound I-treated silica	54	41	27
Benzene–cyclohexane–acetic acid (15:15:0.5)	Compound I-treated silica	32	20	7

The aims of the present research were to investigate the purity of a commercial anthralin sample and to separate, identify, and determine any decomposition products in order to judge the stability and the potency of preparations of the drug as an antipsoriatic agent.

EXPERIMENTAL

Materials—Commercial anthralin NF and reference samples of anthralin, danthron, and dianthrone were used as received[1]. All solvents

[1] Pfaltz and Bauer, Stanford, Conn.

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Table II—UV Spectroscopy Data for Anthralin and Its Decomposition Products

Compound	Maximum Absorbance (λ_{max}), nm	Molar Absorptivity (ϵ)
Anthralin	356	1.03×10^4
Danthron	432	1.19×10^4
Dianthrone	368	3.13×10^4 [a]

[a] The molar absorptivity of dianthrone was calculated on the basis of the molecular weight 450.4 found by Segal *et al.* (17).

were reagent grade and distilled before use. TLC was accomplished on 0.25-mm layers of silica gel 60 GF_{254}[2], precoated plates of the same material, and 0.25-mm layers of silica gel 60 H containing ethylenediaminetetraacetic acid (I) prepared by the method of Arends (11). Column chromatography was accomplished on specially purified silica gel containing 2% I prepared according to the method of Arends (11).

Separation by Column Chromatography—A 500-mg sample of commercial anthralin NF was applied to a column containing 100 g of specially purified silica gel containing I and chromatographed with benzene–cyclohexane–methanol (15:15:0.25). The resulting fractions were examined by TLC. A brown-violet zone, which remained at the top of the column, was eventually eluted with methanol.

Identification of Fractions—*TLC*—Routinely, 5 μl of each fraction was applied to both silica gel TLC plates and I-treated silica gel layers on glass plates, and all plates were developed with either benzene–cyclohexane–methanol (15:15:0.25) or benzene–cyclohexane–acetic acid (15:15:0.5). The plates were air dried, and the spots were identified by color, fluorescence, and R_f values compared with reference samples (Table I).

Melting Point—The three separated compounds were recrystallized from benzene–cyclohexane–methanol, yielding pure characteristic crystals for each compound. The melting points of the obtained compounds were determined[3].

UV Spectroscopy—Solutions (0.001% w/v) of the three isolated compounds in chloroform were prepared. The UV spectra of the solutions were examined[4] from 750 to 350 nm (scan speed of 5 nm/sec), and the absorption maxima (λ_{max}) as well as the molar absorptivity (ϵ) of each compound were determined (Table II).

IR Spectroscopy—IR spectra were obtained for anthralin, danthron, and dianthrone using potassium bromide pellets[5].

Quantitation of Anthralin and Its Decomposition Products—Three random samples of commercial anthralin NF, 50 mg each, were dissolved in chloroform, and the volume of the solution obtained was adjusted to 25 ml in a volumetric flask. A 0.5-ml sample of each solution was diluted to 50 ml with chloroform to give a concentration of 20 μg/ml. These solutions were measured at 356, 432, and 368 nm, corresponding to anthralin, danthron, and dianthrone, respectively. Since the absorption maximum of anthralin interfered with that of dianthrone because of their closeness, it was not possible to determine dianthrone in the presence of anthralin. However, because dianthrone was present in low concentration compared to anthralin, dianthrone did not interfere with the determination of anthralin in the commercial form. It was necessary to separate the mixture into its components by TLC prior to quantitation. A standard absorbance–concentration curve was determined for each compound and was used to estimate its percentage content in the commercial anthralin sample (Table III).

RESULTS AND DISCUSSION

Anthralin is official in the USP and NF. In ointment form at concentrations of 0.1, 0.2, 0.25, 0.4, 0.5, and 5%, it is categorized as a topical antipsoriatic. Topically applied anthralin not only provides a proven efficaceous treatment for psoriasis but is also free from systemic toxicity when used in low concentrations (4, 12–14). Anthralin is preferred over drugs such as steroids, because the prolonged use of steroids commonly induces a facial erythema. Systemic steroids for psoriasis treatment should be avoided whenever possible because they may induce a more severe form of the disease (15).

The degradation products of anthralin constitute one of the most objectionable aspects of anthralin treatment because they are responsible for staining of skin and clothing and irritation of surrounding normal skin

[2] E. Merck and Co.
[3] Thomas-Hoover capillary melting-point apparatus.
[4] Cary model 118 recording spectrophotometer.
[5] Perkin-Elmer 437 grating spectrophotometer.

Table III—Percent of Anthralin and Its Decomposition Products in a Commercial Anthralin Sample

Compound	Amount Found, %	
	TLC Method	Direct Spectrophotometric Method
Anthralin	93.83	95.00
Danthron	4.25	3.75
Dianthrone	1.38	1.25

(2, 8, 16). Few reports deal with the separation and identification of anthralin decomposition products (2, 17, 18), and none deals with the quantitation of these products in anthralin. Segal *et al.* (17) claimed that a commercial quality material of anthralin was purified by TLC on silica gel with benzene. However, since impure anthralin is soluble in benzene, its decomposition products probably are not separated from anthralin with benzene alone as an eluant (19, 20). Thielemann (18) used TLC on silica gel with heptane–benzene–chloroform (1:1:1) as the eluent for separation and identification of anthrarobin and anthralin. The same solvent system also was used by Fisher and Maibach (2) for separation of purified anthralin from its decomposition products, and they identified these products according to the method of Segal *et al.* (17).

TLC—In this study, anthralin and two decomposition products were separated from commercial anthralin (Table I). The two decomposition products were identified as danthron (1,8-dihydroxy-9-anthrone) and dianthrone after matching with reference samples. A very small spot of a violet-colored artifact was noted at the origin on the thin-layer plate. This spot may have resulted from the reaction of traces of iron present in commercial silica gel with anthralin. With column chromatography, a violet-colored zone remained at the top of the column.

The formation of this artifact was prevented either by using salicylic acid-treated silica plates (0.2% w/v in methanol) or by adding salicylic acid to anthralin in a ratio of two parts of salicylic acid to one part of anthralin. The quantity of the artifact was also greatly reduced when I-treated silica gel was used. The salicylic acid may have prevented the formation of the violet-colored artifact by the formation of an iron salicylate, thus effectively removing iron from the presence of anthraquinones. Compound I chelated most of the iron in the I-treated silica, but small quantities of the artifact were observed.

Melting Point—Anthralin was separated as lemon-yellow crystals, mp 181–182° [lit. (21) mp 175–181°]. Danthron was obtained as orange-yellow crystals, mp 193.5–195° [lit. (20) mp 193–197°]. Dianthrone was crystallized as yellow platelets, mp 233–234° [lit. (17) mp 235–237°].

UV Spectroscopy—Table II shows the maximum absorbance (λ_{max}) and the molar absorptivity (ϵ) of anthralin, danthron, and dianthrone in chloroform.

IR Spectroscopy—The IR spectra (potassium bromide) of all three compounds were similar. In the 4000–1700-cm^{-1} region, all three were identical. Minor differences were found in the fingerprint region. All compounds exhibited peaks in the 1635–1640-cm^{-1} region, characteristic of H-bonded C=O conjugated to an aromatic nucleus.

Quantitation of Anthralin and Its Decomposition Products—USP XIX considers danthron as a common constituent of anthralin and offers an equation for the calculation of both compounds in powder or in ointment form. However, danthron reportedly is inactive as an antipsoriatic agent (2, 8, 9) and is partly responsible for the objectionable side effects of anthralin (2, 9, 15).

In this study, two decomposition products for anthralin were found. Therefore, it was necessary to determine each one to judge the potency and, thereby, the therapeutic effectiveness of the drug. Accordingly, a precise determination of anthralin and its decomposition products was accomplished by separating the mixture by TLC prior to the quantitation of each component spectrophotometrically at its own wavelength (Table III). By this method, it was possible to determine quantitatively anthralin, danthron, and dianthrone without any interference or overlapping.

If the original concentration of the commercial anthralin sample is known, it is possible to determine the concentration of the components by direct spectrophotometric measurement. These determinations were made by observing the absorbance at two wavelengths (356 and 432 nm) for anthralin and danthron and then obtaining the amount of dianthrone by algebraic difference (Table III).

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Topical Mosquito Repellents XI: Carbamates Derived from *N,N'*-Disubstituted Diamines

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Abstract □ Carbamates derived from various *N,N'*-disubstituted diamines were synthesized and evaluated as repellents for *Aedes aegypti* mosquitoes with an *in vitro* blood-feeding test system. Several compounds were more effective than diethyltoluamide.

Keyphrases □ Carbamates from *N,N'*-disubstituted amines—synthesized and evaluated as mosquito repellents □ Repellents, mosquito—various carbamates derived from *N,N'*-disubstituted diamines synthesized and evaluated □ Structure–activity relationships—various carbamates derived from *N,N'*-disubstituted diamines evaluated as mosquito repellents

Carbamates as mosquito repellents were discussed previously (1). Substituted 2-oxazolidones were synthesized and found to possess repellent activity (1). However, improvement of their repellency was desired, and a new synthetic effort was initiated to prepare carbamates from *N,N'*-disubstituted diamines.

To vary the boiling-point ranges of the proposed repellents, syntheses were concentrated on ethylenediamine, diethylenediamine, and triethylenediamine derivatives. Previous studies (1) showed that repellency duration is a function of boiling points or volatility of repellents.

These compounds were evaluated in the *in vitro* mosquito blood-feeding test system reported previously (2). Mosquitoes used for these evaluations were female *Aedes aegypti* (yellow fever mosquito).

Table I—Physical and Biological Properties of Carbamates Derived from *N,N'*-Disubstituted Diamines

$R_1N(CH_2CH_2)_nNR_3$ (R_2, R_4)

Compound	R_1	R_2	R_3	R_4	n	Boiling Point (0.5 mm Hg)	Repellency ED_{50}	Repellency ED_{90}	r
I[a]	C_3H_7	H	C_3H_7	CO_2CH_3	1	90°	2.8	79	−0.97
II[b]	C_4H_9	H	C_4H_9	CO_2CH_3	1	102°	0.12	0.59	−0.88
III[c]	C_3H_7	CO_2CH_3	C_3H_7	CO_2CH_3	1	110°	0.049	0.13	−0.92
IV[b]	C_4H_9	CO_2CH_3	C_4H_9	CO_2CH_3	1	130°	0.014	0.072	−0.87
V[d]	C_6H_{13}	H	C_6H_{13}	CO_2CH_3	1	146°	0.028	0.083	−0.79
VI[e]	C_6H_{13}	CO_2CH_3	C_6H_{13}	CO_2CH_3	1	160°	0.036	0.21	−0.84
VII[f]	C_3H_7	H	C_3H_7	CO_2CH_3	2	112°	0.25	2.5	−0.68
VIII[g]	C_3H_7	CO_2CH_3	C_3H_7	CO_2CH_3	2	141°	0.020	0.073	−0.96
IX[h]	C_3H_7	H	C_3H_7	CO_2CH_3	3	131°	0.23	4.0	−0.68
X[i]	C_3H_7	CO_2CH_3	C_3H_7	CO_2CH_3	3	155°	0.018	0.10	−0.82
Diethyltoluamide						100°	0.031	0.10	−0.81
N,N'-Dihexamethylenecarbamide						130°	0.00081	0.056	−0.83

[a] *Anal.*—Calc. for $C_{10}H_{22}N_2O$: C, 59.37; H, 10.98; N, 13.85. Found: C, 58.84; H, 11.03; N, 13.76. [b] See *Experimental.* [c] *Anal.*—Calc. for $C_{12}H_{24}N_2O_4$: C, 55.36; H, 9.29; N, 10.76. Found: C, 55.15; H, 9.23; N, 10.70. [d] *Anal.*—Calc. for $C_{16}H_{34}N_2O_2$: C, 67.08; H, 11.96; N, 9.78. Found: C, 66.99; H, 11.87; N, 9.66. [e] *Anal.*—Calc. for $C_{18}H_{36}N_2O_4$: C, 62.75; H, 10.53; N, 8.13. Found: C, 63.05; H, 10.74; N, 8.23. [f] *Anal.*—Calc. for $C_{12}H_{26}N_2O_2$: C, 62.57; H, 11.38; N, 12.16. Found: C, 62.44; H, 11.54; N, 12.05. [g] *Anal.*—Calc. for $C_{14}H_{28}N_2O_4$: C, 58.30; H, 9.79; N, 9.72. Found: C, 57.99; H, 9.85; N, 9.68. [h] *Anal.*—Calc. for $C_{14}H_{30}N_2O_2$: C, 65.07; H, 11.70; N, 10.84. Found: C, 65.07; H, 11.74; N, 10.66. [i] *Anal.*—Calc. for $C_{16}H_{32}N_2O_4$: C, 60.73; H, 10.19; N, 8.85. Found: C, 60.65; H, 10.39; N, 8.66.

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