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Studies on the Muscarinic and Antimuscarinic Activity of Benzyltrimethylammonium Bromide (BTM)

S. J. STRYCKER* and J. P. LONG

Abstract □ Superfusion of an isolated segment of guinea pig ileum with BTM effectively blocks the contractions induced by histamine, potassium chloride, acetylcholine, and BTM itself for a period of 60–90 min. Spontaneous contractions are also eliminated during this period. Incorporation of atropine in the superfusate removes the blocking activity of BTM, while hexamethonium has no effect on BTM blockade under similar experimental conditions. Perfusion of isolated gut loops of dogs by BTM causes blockade of contractions induced by histamine and acetylcholine. Epinephrine responses were unaffected. However, BTM caused a reduction in the perfusion pressure response to acetylcholine and histamine while the epinephrine response was increased. The pressor response of epinephrine and the depressor response of acetylcholine were significantly reduced in both the systemic and perfused limb blood pressures of the dog following i.m. administration of 1 mg./kg. of BTM.

Keyphrases □ Benzyltrimethylammonium bromide (BTM)—activity □ Muscarinic, antimuscarinic activity—BTM □ Ileum-jejunum, isolated—superfusion technique □ Systemic, perfused limb pressures—BTM effect

The cholinergic properties of benzyltrimethylammonium ion (BTM) were first reported by Alles (1) in 1944. A subsequent study by Lee and Shideman (2) in 1959 showed that BTM exhibited both muscarinic and nicotinic activities in certain preparations, with muscarinic activity predominating. This finding was substantiated by Hamilton and Rubenstein (3) in 1968. They described the dual muscarinic and nicotinic activities of BTM plus its pyridyl analogs. Publications by Wong and Long (4) in 1962 and by Long, Wong, and Witt (5) in 1965 first revealed that the parent compound plus some of its halogenated isomers exhibited anticholinergic activity at higher dosage levels in addition to their cholinergic response. The studies reported in this paper were carried out in an attempt to evaluate the scope of the anticholinergic response and to investigate the extent to which BTM was capable of antagonizing the effects of other agents.

MATERIALS AND METHODS

Isolated Guinea Pig Ileum—The *in vitro* anticholinergic activity was evaluated using the superfused guinea pig ileum (6) obtained from more than 50 animals. The guinea pigs were stunned by a blow on the head, and a 20–30-mm. segment of the lower ileum was removed. The force of contraction of the gut segment was measured with a Statham strain gauge and recorded with an Offner-type RS dynograph. Each ileum preparation was subjected to an initial tension of 1 g., and each gram of force of contraction was calibrated to produce 1.0 cm. of displacement on the record. The ileum strips were constantly superfused with warmed (37°) Tyrode's solution aerated with bubbling 95% oxygen–5% carbon dioxide at a rate of 7 ml./min. by means of a Sigmamotor-type T-8 peristaltic infusion

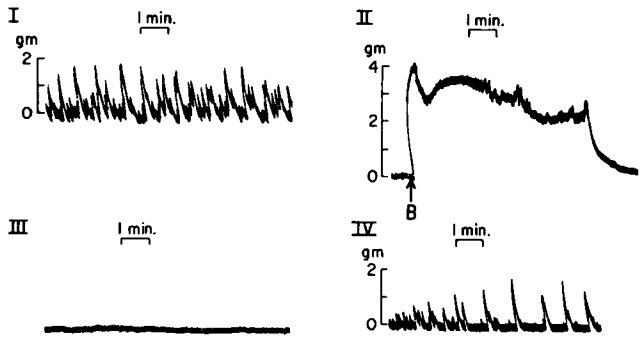

Figure 1—*Typical records obtained with superfused guinea pig ileum (Tyrode's solution). I, spontaneous contracton. II, response obtained upon superfusion of 50 ml. of 10 mcg/ml. of BTM (B) in Tyrode's. III, gut response immediately after BTM superfusion. IV, spontaneous contractions returning after 60–90 min.*

pump. The drugs employed were dissolved in 0.9% saline in appropriate concentrations and were administered *via* a funnel directly onto the ileum segment. BTM was prepared in aerated Tyrode's solution because the intestinal smooth muscle was superfused with up to 50 ml. of the solution containing varying concentrations of BTM. The drugs were administered in random order and were intermittently applied every 5 min. until the contractile responses returned to control values. This preparation is very convenient to study drug interactions of the type outlined in this manuscript. In addition the preparation responds to much lower doses of agonists than is possible with the classical preparation.

Since each segment served as its own control, statistical analyses were performed by the Student *t* test, paired comparisons (7). Values of *p* equal to or less than 0.05 were considered significant.

Benzyltrimethylammonium bromide was prepared according to the method of Baker and Ingold (8), m.p. 234–235°; literature value, 235°. The elemental analyses for BTM–Calculated: C, 52.18%; H, 7.01%; N, 6.09%. Found: C, 52.06%; H, 7.33%; N, 6.10%. Other drugs used were: potassium chloride, acetylcholine chloride, histamine hydrochloride, *l*-epinephrine hydrochloride, morphine sulfate, atropine sulfate, and hexamethonium chloride.

Isolated Small Intestine of Dog—The isolated sections of dog small intestine were prepared as described by Burks and Long (9). Eight mongrel dogs of either sex, weighing 10–13 kg., were anesthetized with 15 mg./kg. of thiopental sodium and 250 mg./kg. of barbital sodium administered i.v. The mesenteric arteries of small (8–10 cm.) sections of small intestine (jejunum-ileum) were cannulated with polyethylene tubing and perfused with warmed (37°) Krebs, bicarbonate solution aerated with bubbling 95% oxygen–5% carbon dioxide. Following perfusion with the salt solution, spontaneous intestinal ativity begins within 5 min. No apparent differences in responsiveness of the two portions of the intestine have been noted. Perfusion was performed with a Sigmamotor-type T-8 peristaltic infusion pump. Perfusion pressure was measured from a T-tube in the cannula between the pump and the artery by a Statham pressure transducer and recorded on an Offner-type RS dynograph. Perfusion pressure was maintained at 80 to 100 mm. Hg. With these experimental conditions a stable preparation is obtained that has a duration of at least 3 hr.

The mesenteric vein of the gut section was also cannulated to allow uniform drainage. The intestinal segment was ligated on both sides of the perfused section and was surgically excised, placed on warmed, saline-soaked gauze pads and maintained at 37° by use of an incandescent lamp. A latex balloon was placed in the lumen of the intestinal segment for measurement of intraluminal pressure; the water-filled balloon was attached to a Statham pressure transducer and the pressure recorded on an Offner recorder. With this preparation one can evaluate drug activity on the resistance vessels of the intestine as well as tone of the intestinal muscle. The sympathetic nerves innervating the vessels and intestinal muscle can be isolated and stimulated electrically.

The drugs employed were dissolved in 0.9% saline in appropriate concentrations and were administered *via* the arterial cannula in random order. Once control responses were obtained, 80 ml. of 20 mcg./ml. of BTM in Krebs' solution was infused to establish the blockade. In this preparation infusion of large amounts of normal saline will decrease vascular reactivity. The drugs were administered once each for a 10-min. period until responses returned to control values. Thus, each segment served as its own control. However, when morphine was used, separate segments were utilized for control and for post-BTM treatment.

Systemic and Perfused Limb Pressures—Five mongrel dogs of either sex weighing between 9 and 13 kg. were anesthetized with 15 mg./kg. of thiopental sodium and 250 mg./kg. of barbital sodium administered i.v. The trachea was cannulated, and the animal was artificially respired with a Palmer respirator when it proved necessary. Bilateral vagotomies were performed. Measurements of the systemic pressure were made from the cannulated left carotid artery by means of a Statham pressure transducer and Offner recorder. Blood from the cannulated left femoral artery was pumped through heparinized Tygon tubing at constant pressure to a cannula in the right brachial artery by means of a Sigmamotor peristaltic infusion pump. The major collaterals of the brachial artery were isolated and ligated and the innervation to the limb was sectioned. A T-tube between the pump and the brachial artery was connected to a Statham pressure transducer and an Offner recorder in order to monitor the perfused limb pressure. Since the flow was maintained at a constant level, changes in pressure reflect changes in resistance. Drugs administered to the perfused limb were injected into the tubing which lead from the left femoral artery. For systemic administration, the drugs were injected into the isolated right femoral vein.

Once reproducible control responses were obtained, 1 mg./kg. of BTM was administered i.m. into the right hind limb. The i.v. route was contraindicated, because it caused a cardiovascular crisis. Responses to the drugs were then obtained post-BTM administration. Again, each animal served as its own control. The Student *t*-test, paired comparison test was used for statistical analysis.

RESULTS

The superfused guinea pig ileum proved to be an excellent preparation for the evaluation of several drugs simultaneously. The gut segment responded uniformly to very low concentration of agonists; the preparation provided a distinct advantage in ease of administration; and each gut segment could serve as its own control. Figures 1 and 2 illustrate typical records obtained.

Superfusion of the gut segment with a standard dose of 50 ml., 10 mcg./ml., of BTM clearly diminished the spontaneous contractions and, in addition, significantly blocked the stimulating responses to histamine, acetylcholine, potassium chloride, and to BTM itself. The onset of blockade was immediate, following superfusion, and maximal blockade developed within 20–30 min. following the end

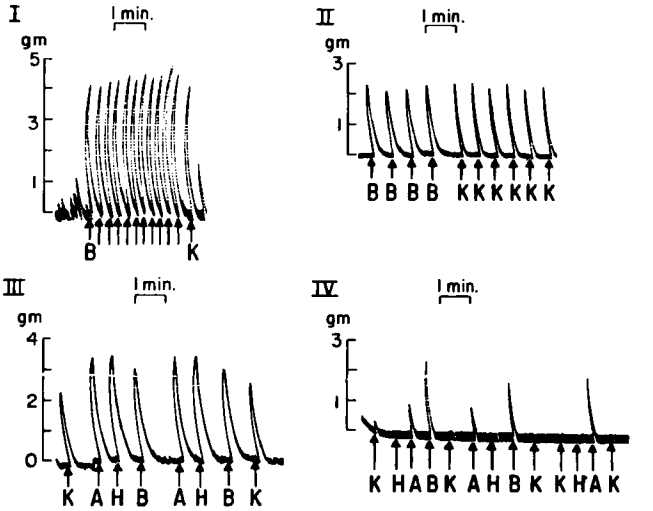

Figure 2—*Agonist-induced contractions of the superfused ileum before and after BTM superfusion. I, repeated BTM (B) administration followed by KCl (K) administration. II, repeated BTM (B) followed by repeated KCl(K) administration. III, consecutive agonist administration pre-BTM superfusion. K = KCl, A = acetylcholine, H = histamine, B = BTM. IV, consecutive agonist administration 30 min. post-BTM superfusion. Doses of agonists: KCl, 400 mcg.; BTM, 1 mcg.; histamine, 0.1 mcg.; ACh, 0.01 mcg.*

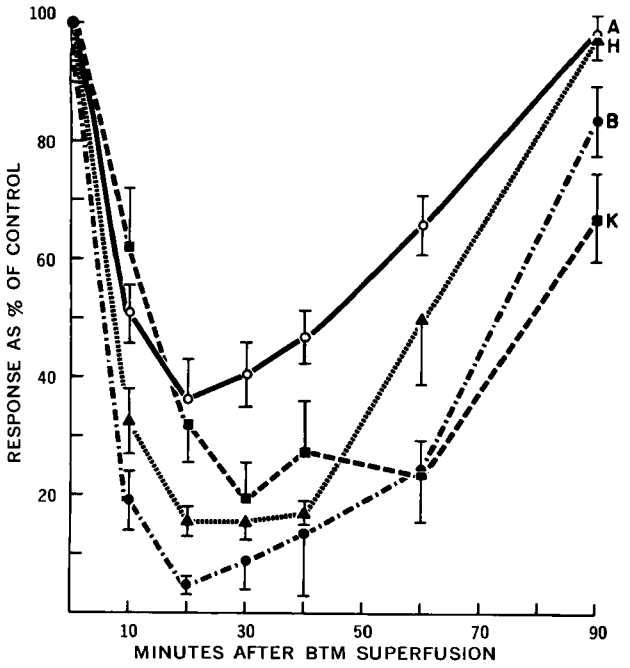

Figure 3—*The decreased response of the ilea segments to agonists following BTM superfusion. Control values* ± SE *in grams tension developed: A = acetylcholine = 2.5 ± 0.2; H = histamine = 3.4 ± 0.1; B = benzyltrimethylammonium = 2.4 ± 0.2; K = potassium chloride = 2.2 ± 0.2. All values are statistically significant at* $p < 0.05$ *from 10 through 60 min., while KCl is significant through 90 min (n = 10).*

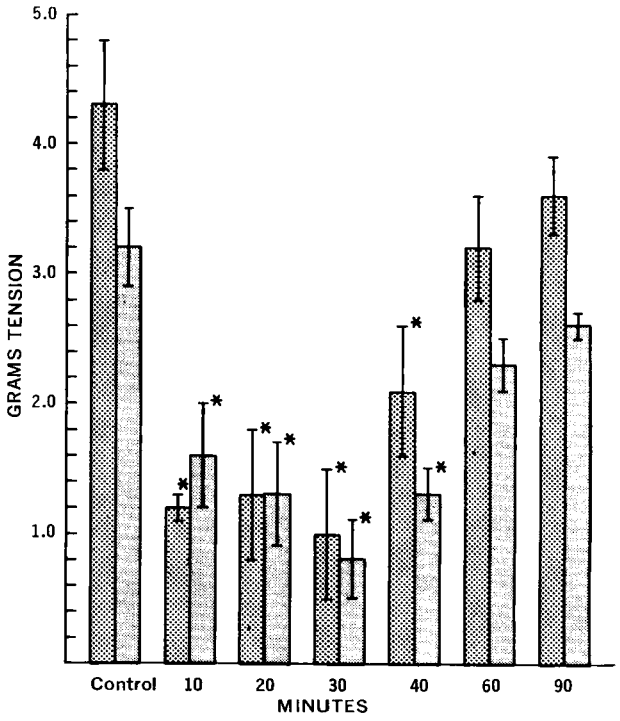

Figure 4—*Response of ilea segments post-BTM superfusion. Atropine, 20 ml. of 0.2 mcg./ml., administered during the period of 15–20 min. following BTM superfusion. Key:* ▨, *KCl;* ▤, *histamine.* *$p < 0.05$; *n = 4.*

of the 10 mcg./ml. BTM superfusion. Responses gradually returned to control values within 90 min., with the exception of KCl which required 120 min. (Fig. 3). Recovery from the blockade was normally presaged by the appearance of spontaneous contractions.

In another series of experiments using 10 animals, muscarinic receptors were implicated by the incorporation of atropine in the Tyrode's superfusate at 0.2 mcg./ml. throughout the experiment. No contractile responses were observed by the administration of BTM or acetylcholine, although spontaneous contractions were vigorous. However, normal contractile responses were obtained from KCl and histamine administration both before and after administration of the standard blocking dose of BTM. In fact, the observed trend suggested that these two agonists were slightly more active following atropine after BTM superfusion.

In another series of experiments 20 ml. of 0.2 mcg./ml. of atropine was administered during the period of maximum blockade, 15-20 min. following the end of BTM superfusion. Under these conditions, responses to the agonists returned to control values within 60 min. instead of 90 min. as before (Fig. 4). In a separate experiment using five segments the incorporation of hexamethonium at 10 mcg./ml. in the Tyrode's had no observable effect on the responses of any of the agonists.

Isolated Small Intestine of the Dog—Both perfusion and contraction pressures were monitored in this preparation (Table I). The anticholinergic activity of BTM was again demonstrated after perfusion of a blocking dose of BTM (80 ml. of 20 mcg./ml.). The contractile responses of the agonists BTM, ACh, and histamine were decreased significantly for a period of up to 30 min. following the BTM perfusion. The normally relaxing response of epinephrine did not appear to be affected.

However, the effect of BTM perfusion on the response of the musculature of the mesenteric artery supplying the loop to these agonists was marked. Perfusion pressure on administration of BTM, ACh, and histamine was decreased, while epinephrine exhibited a significant increase.

Spasms of the gut segment induced by the administration of 50 mcg. of morphine were also affected by the prior perfusion of BTM (Fig. 5). Due to the duration of the effect of morphine, controls were obtained on separate segments. BTM was perfused and morphine was administered within 5 min. The response was arbitrarily analyzed by comparing the number of contractions during a 10-min. interval both pre- and post-BTM perfusion (pre-BTM, 83.3 ± 3.0; post-BTM, 35.4 ± 3.2). More significantly, the force of contractions was also decreased by the action of BTM.

Table I—Pressure Changes in mm. Hg ± *SE* Produced by Drug Administration During the Indicated Time Intervals (Minutes) Following BTM Perfusion

Drug[a]	Control	0–10	10–20	20–30	30–40	40–50
			Perfusion Pressure at Various Time Intervals			
ACh	23.5 ± 1.1	7.7 ± 1.2[b]	10.7 ± 3.3[b]	11.1 ± 2.1[b]	15.1 ± 2.9[b]	14.6 ± 4.3
Histamine	14.1 ± 1.8	2.0 ± 0.2[b]	2.0 ± 0.2[b]	5.0 ± 1.7[b]	4.4 ± 0.2[b]	2.2 ± 1.2[b]
Epinephrine	55.1 ± 5.1	51.9 ± 6.7	73.1 ± 9.6[b]	80.0 ± 10.9[b]	80.8 ± 11.9[b]	81.7 ± 4.4[b]
BTM	34.0 ± 6.4	23.0 ± 6.4	21.0 ± 6.6[b]	20.5 ± 6.1[b]	42.5 ± 16.4	38.6 ± 13.5
			Intraluminal Pressure at Various Time Intervals			
ACh	63.3 ± 3.9	1.1 ± 0.1[b]	21.3 ± 12.7[b]	32.8 ± 12.7[b]	49.3 ± 13.2	53.5 ± 17.0
Histamine	53.0 ± 7.9	2.6 ± 0.5[b]	2.6 ± 0.5[b]	1.6 ± 1.6[b]	22.2 ± 12.2[b]	22.2 ± 17.0
Epinephrine	2.7 ± 0.2	1.1 ± 0.1	2.3 ± 0.3	2.8 ± 1.1	3.3 ± 1.4	2.3 ± 0.1
BTM	64.4 ± 5.1	12.0 ± 7.2[b]	17.5 ± 7.5[b]	44.3 ± 8.7	53.0 ± 9.6	56.0 ± 10.9

[a] Doses used were: BTM (benzyltrimethylammonium), 10 mcg.; histamine, 2 mcg.; ACh (acetylcholine), 0.2 mcg.; epinephrine, 1 mcg. [b] Statistically significant at the $p < 0.05$ level (N = 7).

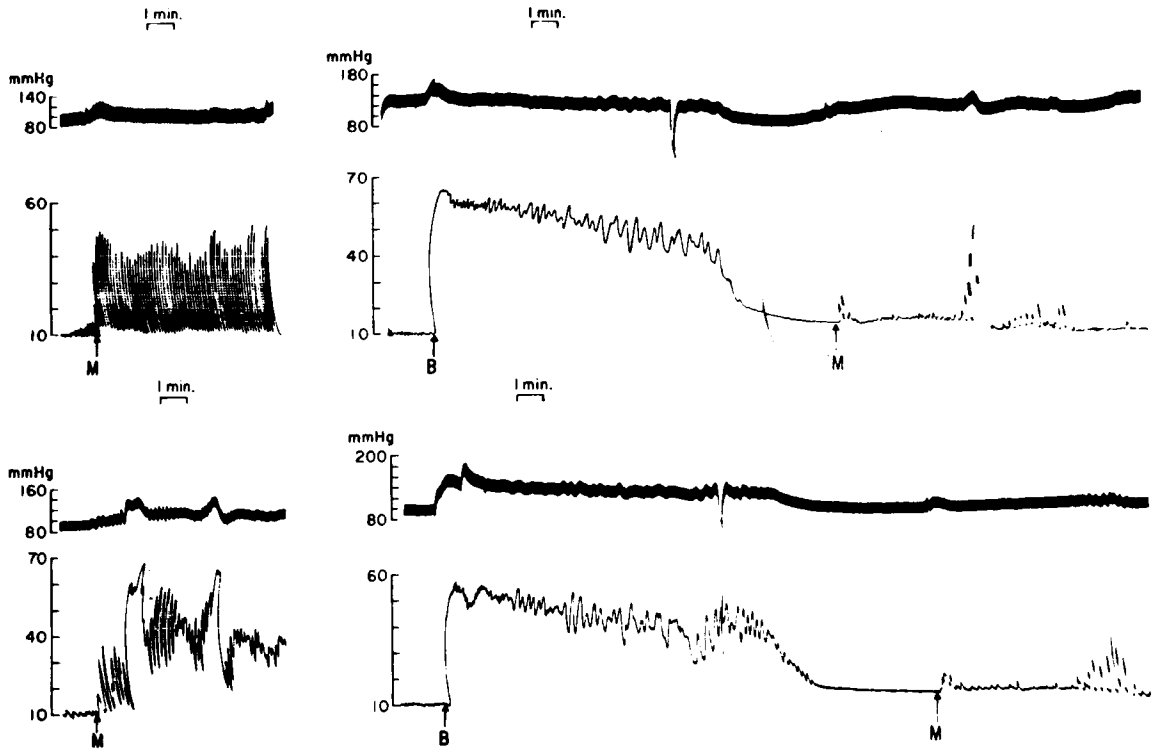

Figure 5—*Responses of two isolated dog gut loops to 50 mcg. of morphine (M) prior to and following 80 ml. of 20 mcg./ml. of BTM (B) infusion. Top records are perfusion pressure and bottom records are from intestinal contracture. Left portion of figure is control and right portion is experimental.*

Isolated Limb and Systemic Blood Pressures—The anticholinergic action of BTM, as observed in the systemic blood pressure preparation with the dog, was reported previously (5). These results with acetylcholine were confirmed and extended to the blood pressure of the isolated limb. In addition, the effect of 1 mg./kg. of BTM administered i.m. on the response of systemic and perfused limb pressures to epinephrine was recorded. The effects of acetylcholine and epinephrine were antagonized by BTM in both instances (Table II).

DISCUSSION

The results of these experiments illustrate the nonspecificity of the anticholinergic activity of BTM. BTM exhibited the ability to inhibit the contractions induced by histamine and potassium chloride in the guinea pig ileum preparation, as well as acetylcholine and BTM itself. Incorporation of atropine in the Tyrode's superfusate completely blocked the contractions due to BTM and acetylcholine and the presence of atropine prevented BTM from antagonizing KCl or histamine. The duration of the blockade, 60 to 90 min., coupled with the repeated challenges by the various agonists suggest that the inhibition is of a noncompetitive type. In addition to its probable inhibition of the muscarinic receptor as suggested by Long *et al.* (5), BTM is possibly disturbing the ion balance at the synapse.

The calcium ion has been implicated by others in an attempt to explain the lack of contraction in vascular smooth muscle under controlled experimental conditions. For instance, it has been demonstrated that Ca^{++} deficiency produces a nonspecific depression of dog mesenteric arteries to sympathetic nerve stimulation and to administered epinephrine, norepinephrine, 5-hydroxytryptamine, and angiotensin (10). Since Ca^{++} depletion of perfused tissues is usually considered to require 30–90 min. (11), the immediate inhibition by BTM in both the superfused ileum and perfused dog gut loop requires further explanation.

Calcium ion was also implicated in the blockade of contractions of the taenja coli of guinea pigs caused by potassium chloride, angiotensin, and acetylcholine by the administration of the adrenergic blockers: chlorpromazine, dibenamine, or phenoxybenamine (12). Measuring ^{45}Ca uptake demonstrated that the adrenergic blockers reduced the Ca^{++} uptake only for potassium chloride and acetylcholine. High external calcium ion concentration reversed only the block of potassium induced contractions.

Thus, it is conceivable that BTM blockade of smooth muscle contractions is by a dual mechanism: by a noncompetitive blockade of the muscarinic receptor and by calcium-ion depletion. However, the significant increase in perfusion pressure in the dog gut loop preparation by epinephrine administration following BTM perfusion does not support this hypothesis. The effect of BTM on smooth muscle vasculature may involve a more subtle modification of the sodium ion-potassium ion balance.

Table II—Pressure Changes in mm. Hg ± *SE* Produced by Drug Administration both Pre- and Post-BTM

Drug	Perfused Limb Pressure[a]		Systemic Pressure[b]	
	Pre-BTM	Post-BTM	Pre-BTM	Post-BTM
Epinephrine	+50.8 ± 5.3	+35.9 ± 5.6[c]	+57.5 ± 10.4	+20.6 ± 5.4[c]
Acetylcholine	−24.6 ± 1.0	−8.5 ± 1.4[c]	−32.4 ± 4.3	−4.3 ± 1.8[c]

[a] Perfused limb doses were: epinephrine, 1 mcg. and acetylcholine, 2 mcg. [b] Systemic doses were: epinephrine, 1 mcg./kg., acetylcholine, 2 mcg./kg. BTM was administered at 1 mg./kg. i.m. [c] Denotes significance at the $p < 0.05$ level (N = 8).

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Coumarins IX: Coumarins of *Sphenosciadium capitellatum* (A. Gray)

KUO-HSIUNG LEE* and T. O. SOINE†

Abstract □ The aliphatic naphtha extract of the roots of *Sphenosciadium capitellatum* (A. Gray) has yielded isoimperatorin, phellopterin, imperatorin, oxypeucedanin, isopimpinellin, and a linear furanocoumarin, $C_{17}H_{16}O_5$, which has been assigned the 5-γ,γ-dimethylallyloxy-8-methoxy psoralen structure on the basis of degradative and spectral studies. This linear furanocoumarin appears to be identical to cnidilin, recently isolated and characterized from *Cnidium dubium* (Schkuhr) Thell. The methanol extract yielded sucrose and the glycosidic coumarin, nodakenin, in excellent yield.

Keyphrases □ Coumarins—*Sphenosciadium capitellatum* □ *S. capitellatum* roots—coumarin extraction □ Column chromatography—separation □ Paper chromatography—separation, identity □ IR spectrophotometry—structure, identity □ UV spectrophotometry—structure, identity □ NMR spectroscopy—structure, identity □ Mass spectroscopy—molecular weight

Examination of the coumarinic content of the roots of *Sphenosciadium capitellatum* (A. Gray) is a continuation of a general program (1–5) for investigating umbelliferous plants for agents with potentially useful physiological activity (6). Of further interest was the fact that this particular species is the sole representative of the *Sphenosciadium* genus. The extraction of the coumarins was carried out by using aliphatic naphtha,[1] ether, and methanol, respectively. The aliphatic naphtha extract was column-chromatographed over silica gel and yielded a number of known linear furanocoumarins, *i.e.*, isoimperatorin (I), phellopterin (II), imperatorin (III), oxypeucedanin (IV), and isopimpinellin (V). In addition, an apparently undescribed furanocoumarin (VI) was obtained.

The ethereal extract was not investigated further since it showed virtually identical components when compared by TLC with the aliphatic naphtha extract.

Compound VI, m.p. 115–115.5°, shows a molecular ion peak at *m/e* 300 in the mass spectrum and is optically inactive. Its elementary analysis agrees with a molecular formula of $C_{17}H_{16}O_5$ and it appears as a single fluorescent yellow spot under UV light by TLC on Silica gel G in three different solvent systems.[2] The IR spectrum indicated that VI is a furanocoumarin (7).

The UV spectrum of Compound VI is virtually superimposable upon that of phellopterin (II) (m.p. 101–102°) (7). It remained unchanged on the addition of sodium hydroxide, indicating the absence of a free phenolic hydroxy group. The possibilities for the existence of an angular system like that of either VII or VIII for the structure of Compound VI could be ruled out since an angular furanocoumarin would show a completely different UV spectrum compared with that of the corresponding linear one (7).

Compound VI displayed NMR signals at 8.33 (3H, s), 8.22 (3H, s) ($=C\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}$), 5.87 (3H, s, $-OCH_3$), 5.21 (2H, d, J = 7 c.p.s., $-CH_2-$), 4.44 (1H, t, J = 7 c.p.s., $-CH=$), 3.74 (1H, d, C_3-H, $J_{3,4}$ = 10 c.p.s.), 1.85 (1H, d, C_4-H, $J_{3,4}$ = 10 c.p.s.), 3.07 (1H, d, C_4'-H, $J_{4',5'}$ = 2 c.p.s.) and 2.38τ (1H, d, $C_5'-H$, $J_{4',5'}$ = 2 c.p.s.) and is in fact also identical with that of phellopterin (II) except for the splitting of the gem-dimethyl group into a doublet instead of the singlet which is found in the latter (7).

The assignment of a γ,γ-dimethylallyloxy group as the C_5-substituent in place of a γ,γ-dimethylallyl group was quite apparent from the evidences of its elementary

[1] Skellysolve B, Skelly Oil Co., Kansas City, Mo.

[2] *a*, Skellysolve B–ethyl acetate = 3:1; *b*, skellysolve B–benzene–methanol = 5:4:2; *c*, ethyl acetate–a mixture of 2:1 dichloromethane–carbon tetrachloride = 8:92.