stretching band could not be located and was presumably submerged in the large C—H stretching band.)

If the proposed structure for the complex is correct, it follows that methylation of either the —NH or —OH group (or both) of acetaminophen should prevent complexation with antipyrine. This was shown to be the case by melting-point determinations; neither congruently nor incongruently melting complexes were formed by the methylated derivatives of acetaminophen with antipyrine (Table II). The literature reports of lack of complexation between antipyrine and acetanilide or phenacetin (*p*-ethoxyacetanilide) were also confirmed.

It is perhaps surprising that since phenol forms a complex with antipyrine (4), *p*-hydroxy-*N*-methylacetanilide does not. The reason for this, which could be confirmed unequivocally only by X-ray crystallography, probably lies in the bulky nature of the acetamido group forcing a different crystal structure on the complex with antipyrine. Thus, *p*-hydroxy-*N*-methylacetanilide, because of its lack of a —NH group, is unable to complex in the way acetaminophen does; and by virtue of its steric properties, it cannot complex like phenol.

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Oral Absorption of ^{14}C-Labeled Mepenzolate Bromide in Humans

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Abstract □ In a pilot study, a single oral dose of 25 or 30 mg. of mepenzolate bromide containing approximately 24 μc. of ^{14}C was given to each of four human volunteers and elimination of ^{14}C was followed in urine and feces for 4 or 5 days. From either a solid (capsule) or liquid dosage form, an average of 14% was eliminated in the urine (capsule: 7.0–20.2%, liquid: 3.1–21.1%), indicative of substantial oral absorption for an onium compound.

Keyphrases □ Mepenzolate bromide, radiolabeled—oral absorption, humans □ Absorption, oral—radiolabeled mepenzolate bromide, humans □ Radiolabeling—preparation of ^{14}C-mepenzolate bromide

It is well known that, as a generality, highly ionized organic compounds are not well absorbed following oral administration (1), and this appears true for some quaternary ammonium spasmolytic agents (2–4).

Mepenzolate bromide[1] (3-hydroxy-1,1-dimethylpiperidinium bromide benzilate), a potent postganglionic parasympathetic inhibitor (5), was reported (6) to be clinically useful in the treatment of motility disorders of the small and large bowels. Since no data have been reported on the oral absorption of this agent in humans, the present pilot study was undertaken to determine the absorption of a single oral dose as determined by excretion of ^{14}C in the urine. The assumption was made that little, if any, hydrolysis would occur in the intestinal tract. For this purpose, the compound was synthesized with a ^{14}C-label in one of the methyl groups. An additional objective was to compare the degree of absorption of the compound in solid dosage form (capsule) with that of a liquid solution formulation.

EXPERIMENTAL

Preparation of ^{14}C-Mepenzolate Bromide—*N*-Methyl-3-piperidyl benzylate, a commercial intermediate[2], was purified from water-insoluble material by solution in dilute hydrochloric acid, filtration with charcoal, and precipitation with dilute ammonium hydroxide. The solid was crystallized from hexane to form lustrous white crystals, m.p. 99–100°. Trial reactions of this tertiary amine with methyl bromide in acetone indicated almost quantitative formation of the insoluble onium salt in approximately 5 days at room temperature, m.p. 229–230° uncorr. [lit. (7) m.p. 234–236°]. Adequate pure tertiary amine was allowed to react in acetone with freshly prepared ^{14}C-methyl bromide[3]. The labeled product melted at 230–231° uncorr. and gave an IR spectrum identical to that of authentic cold material. Specific activity was 4.87 μc./mg. TLC [*n*-butyl al-

[1] Cantil, Lakeside Laboratories, Milwaukee, Wis.

[2] Supplied by Dr. Claude Judd, Lakeside Laboratories, Milwaukee, Wis.

[3] This reaction was carried out by Mallinckrodt-Nuclear, St. Louis, Mo., since ^{14}C-methyl bromide is prepared only on special order.

Table I—Cumulative Total ^{14}C Excretion Data, Urine Plus Feces, in Percent

Volunteer	Day	Capsule Urine	Capsule Feces	Capsule Total	Liquid Urine	Liquid Feces	Liquid Total
1	1	11.0	14.4	25.4	15.9	—	15.9
	2	13.6	46.7	60.3	18.6	0.5	19.1
	3	15.5	46.7	62.3	21.9	58.2	80.1
	4	17.2	55.3	72.5	24.1	—	82.3
	5	—	57.6	74.8	25.1	—	83.3
2	1	15.0	0.1	15.1	2.2	45.8	47.9
	2	18.7	43.0	61.7	2.8	49.8	52.6
	3	19.6	56.5	76.1	3.1	50.6	53.7
	4	20.2	59.3	79.5	3.1	50.7	53.8
	5	—	59.3	79.5	3.1	50.7	53.9
3	1	4.8	0.0	4.9			
	2	6.0	52.1	58.1			
	3	6.5	52.1	58.6			
	4	7.0	59.9	66.9			
	5	—	60.0	67.0			
4	1				4.7	56.2	60.9
	2				5.6	93.5	99.0
	3				5.9	94.9	100.9
	4				6.3	95.8	102.0
	5				6.4	96.0	103.3
5	1	10.2	—	10.2			
	2	12.7	50.5	63.1			
	3	14.6	50.5	65.1			
	4	14.7	50.5	65.2			
	5	—	52.5	67.2			
6	1				16.6	0.1	16.7
	2				20.3	83.3	103.6
	3				21.4	83.6	104.9
	4				21.9	84.1	106.0
	5				22.2	84.2	106.3
Average	Day 1	10.3			9.9		
Urinary ^{14}C	Total	14.8			14.2		

cohol–acetic acid–water (4:1:5) v/v/v] on an EK-6060 silica gel plate showed only a single peak when scanned[4] for radioactivity.

Preparation of Dosage Forms—*Capsules*—Into each of four capsules there was weighed approximately 5.0 mg. of ^{14}C-mepenzolate bromide (5.08, 5.04, 5.09, and 5.12 mg.), followed by 20.0 mg. of cold mepenzolate bromide.

Liquid—To 25.0 ml. of a liquid formulation containing 125.0 mg. of cold mepenzolate bromide[5], there was added 25.0 mg. of ^{14}C-mepenzolate bromide to give a solution containing a total of 30.0 mg./5 ml.

Medication—*Capsules*—Four healthy, consenting adult males received one capsule each in the morning under medical supervision (observed for 4 hr. after medication). Total urinary output was collected for the next 4 days (urines were pooled each day), and total feces were collected for 5 days. Plasma samples were drawn at 0, 2, 4, 8, 24, and 48 hr.

Liquid—This portion was performed 4 months after the capsule study. Two of the volunteers were the same. Each of the four consenting adult healthy males drank a solution prepared from 5.0 ml. of the liquid preparation dissolved in 100 ml. of water plus several washings to ensure complete transfer. Total urinary and fecal outputs were collected for the next 5 days (urines were pooled each day).

Measurement of Radioactivity—A toluene-based fluid was used for counting in a liquid scintillation counter[6]. All values were corrected for quenching by way of external standard channel ratios based on a series of previously prepared quenched samples. Duplicate counting of the ^{14}C-mepenzolate and of some of the urine and plasma samples was done in a separate instrument[7] with good agreement. A 0.20-ml. aliquot of each day's urine was used for measurement. Feces were homogenized with water; a 100–300-mg. aliquot was combusted in a modified Griffiths–Mallinson (8) apparatus, and the trapped $^{14}CO_2$ was counted[8]. Plasma aliquots were treated as urine, except for a few samples which were additionally combusted and measured as $^{14}CO_2$.

RESULTS AND DISCUSSION

Before studies were performed in humans, ^{14}C-mepenzolate bromide from the same batch used in the human study was supplied for a study in rats and dogs. The report of the results of these studies[9] showed that no radioactivity remained in the carcasses of rats 14 days after a single oral dose of 4–5 mg./kg. of ^{14}C-mepenzolate bromide; essentially 100% of the radioactivity was accounted for in the total of urine and feces collected during the period. A single oral dose of 1.5–2.0 mg./kg. to dogs indicated approximately 20% excretion of ^{14}C in the urine (7 days of collection). As will be seen, this approximated the highest human values for urinary ^{14}C excretion.

The results in humans are summarized in Table I. Two volunteers were common to both studies. Total urinary ^{14}C excretion following ingestion of the capsules ranged from 7.0 to 20.2% (average 14.8%) and from 3.1 to 21.1% (average 14.2%) for the liquid.

Total recovery of radioactivity in urine plus feces varied from approximately 100% (two individuals) to 54 and 67% (three individuals), with the other three values between these. In spite of great care (professed by the volunteers), it is impossible to know if total collection of urine and feces were always achieved.

The data of the table indicate that the majority of ^{14}C excretion occurs in the first 24 hr. after ingestion; however, excretion during the 2nd day is appreciable and small amounts are evident after 4 or 5 days. Counts obtained from plasma samples were too close to background values to be meaningful and are not reported; a similar result was reported for plasma values in a study with radioactive butylscopolammonium bromide (9).

The data of this report are indicative of similar oral absorption of ^{14}C for the capsule or liquid dosage form and for higher oral absorption than that reported for anisotropine methylbromide or propantheline bromide (2, 3) if the assumption is made that the ^{14}C excretion represents mepenzolate bromide absorbed. The data additionally suggest that further clinical evaluation should be performed with multiple doses of mepenzolate bromide; since mepenzolate bromide is 25–50% as potent as atropine (7) as a spasmolytic agent[10], it is conceivable, based on the absorption data, that with the recommended clinical dose (25 or 50 mg. t.i.d.) adequate amounts for good pharmacological action will be made available. Information on metabolism, however, is necessary to clarify this point, because the present study may not evaluate the intact mepenzolate bromide molecule.

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[6] Beckman model LS-250, courtesy of Dr. J. Hill, Lakeside Laboratories, Milwaukee, Wis.
[7] Packard Tri-Carb scintillation spectrometer, model 3310, courtesy of Dr. Deane Calvert, Department of Pharmacology, Medical College of Wisconsin.
[8] Courtesy of Dr. J. Hill, Lakeside Laboratories, Milwaukee, Wis.
[9] The authors thank Dr. Claude Judd and Dr. J. Hill for the reports of the results of these studies performed at Lakeside Laboratories.
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A Phytochemical Investigation of *Yucca schottii* (Liliaceae)

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Abstract □ The saponin-containing fraction of the leaves of *Yucca schottii* Engelm. (Liliaceae) has been shown to possess anti-inflammatory properties against carrageenin-induced edema in rats. By means of silica gel G dry column and thin-layer chromatographic techniques, a separation of the saponins present as the acetates was accomplished. Six of the saponins showed the presence of yuccagenin as their aglycone and galactose as the sugar moiety. The other saponin contained kammogenin as its aglycone and 2-deoxyribose as the sugar moiety. A preparative isolation procedure was developed for the saponin which contained kammogenin as its aglycone. It was shown to contain 5 units of 2-deoxyribose attached to the 3-position of the aglycone.

Keyphrases □ *Yucca schottii* (Liliaceae)—phytochemical investigation, saponin fraction constituents, potential anti-inflammatory agents □ Saponins—from *Yucca schottii* leaves, separation, potential anti-inflammatory agents □ Anti-inflammatory properties—constituents of leaves of *Yucca schottii* □ Medicinal plants—*Yucca schottii* leaves, constituents, anti-inflammatory properties

In a continuing search for the anti-inflammatory substances from plant sources, the saponin-containing fraction of the leaves of *Yucca schottii* Engelm. (Liliaceae) has shown activity in a preliminary screen utilizing a procedure (1) involving carrageenin-induced edema in rats. The plant[1] material used for this investigation was collected in Pima County, Ariz., during May 1967.

A preliminary examination of the *n*-butanol extract revealed the presence of saponins. The partial resolution of seven detectable saponins as their acetates was effected utilizing a modified dry column chromatographic separation (2). Further purification of the saponin acetates obtained from the column was achieved using TLC. The saponins were then regenerated by alkaline hydrolysis of the respective acetates.

Each of the seven saponins was acid hydrolyzed into its aglycone and sugar moiety. The aglycone of saponins I–V and VII was identified as yuccagenin. The sugar moiety associated with each saponin was identified as galactose by means of TLC and GLC of the trimethylsilyl ethers. Saponin VI showed a ketone group as indicated by the IR spectrum. Its aglycone was identified as kammogenin. The sugar moiety associated with it was identified as 2-deoxyribose.

Saponin acetate VI was separated from the other acetates by means of Girard's reagent "T" (3). The Girard derivative was acid treated, followed by alkaline hydrolysis to yield saponin VI. An elemental analysis[2] indicated that saponin VI contained 5 moles of 2-deoxyribose and its molecular weight was 1025, corresponding to the molecular formula $C_{52}H_{80}O_{20}$. Methylation of saponin VI and subsequent acid hydrolysis yielded 2-methoxykammogenin. The glycosidic linkage occurs at the 3-position of kammogenin, as shown by the keto-derivative which was obtained by means of an Oppenauer oxidation (4, 5) and also by Killiani oxidation and isomerization (6).

EXPERIMENTAL[3]

Extraction—Five kilograms of the dried leaves of *Y. schottii* was macerated with approximately 4 l. of hot distilled water for 3 hr. The aqueous extract was filtered and evaporated to approximately 25% its volume. The marc was discarded. The aqueous extract was then exhaustively extracted with *n*-butanol. The butanol extract was washed with water saturated with butanol until the Benedict reagent test for reducing sugars was negative. The butanol extract was evaporated *in vacuo* until completely dry; 180 g. of essentially pure sugar-free saponins were obtained. The yield was 3.6% from the dried plant.

TLC analysis of portions of the saponin-containing extract was performed by allowing the lower phase of a chloroform-methanol-water (65:25:10) mixture (7) to ascend, for 24 hr., plates of silica gel G[4], with a piece of Whatman No. 1 paper attached to their top and folded over their backs in order to extend the development time of

[1] Identification confirmed by Robert J. Barr, College of Pharmacy, and Dr. Charles Mason, Botany Department, University of Arizona, Tucson, Ariz. A reference specimen was also deposited in the University of Arizona Herbarium.

[2] Carbon and hydrogen determinations performed by Huffman Laboratories Inc., Wheatridge, Colo.

[3] Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. TLC was carried out on unactivated silica gel G with a stationary phase thickness of 0.3 mm. UV spectra were determined in ethanol using a Beckman DB spectrophotometer. IR spectra were run using KBr pellets on a Perkin-Elmer infracord model 137. Mass spectra were obtained from a Hitachi Perkin-Elmer double-focusing spectrometer, model RMU-6e. GLC analyses were carried out on a Perkin-Elmer gas chromatograph model 880, using 2.5% SE-30 as stationary phase.

[4] Merck.