lizing effect of sorbitan monooleate on salicylic acid may account for its higher rate of diffusion from bases containing this surfactant (Figs. 2–4). However, at the higher drug concentration, a relatively greater proportion of salicylic acid particles could not be effectively surrounded by the nonfused base; therefore, the increase in diffusion rate was apparently due mainly to this factor, which would probably outweigh the surfactant effect.

Figures 1–3 also show that greater drug concentration produced higher rates of diffusion in both methods used to make the ointments. This is in agreement with the results obtained by Lockie and Sprowls (9) who found that the rate of diffusion of iodine and sulfathiazole from ointments increased with increasing drug concentration within a certain range. Undoubtedly, the solubility of a drug in an ointment base plays a major role in the diffusion or release of the drug from the base (10). The faster diffusion demonstrated by bases with higher concentrations of salicylic acid apparently reflects this solubility effect. The higher the concentration of salicylic acid, the more will be left undissolved and not effectively coated by the base.

REFERENCES

(1) C. W. Whitworth, *J. Pharm. Sci.*, **57**, 1540(1968).

(2) C. W. Whitworth and R. E. Stephenson, *ibid.*, **60**, 48(1971).

(3) M. Nakano and N. K. Patel, *ibid.*, **59**, 985(1970).

(4) J. F. Stark, J. E. Christian, and H. G. DeKay, *J. Amer. Pharm. Ass., Sci. Ed.*, **47**, 223(1958).

(5) K. C. Patel, G. S. Banker, and H. G. DeKay, *J. Pharm. Sci.*, **50**, 300(1961).

(6) R. E. Dempski, J. B. Portnoff, and A. W. Wase, *ibid.*, **58**, 579(1969).

(7) J. A. Wood, L. W. Rising, and N. A. Hall, *ibid.*, **51**, 669(1962).

(8) H. B. Kostenbauder and A. N. Martin, *J. Amer. Pharm. Ass., Sci. Ed.*, **43**, 401(1954).

(9) L. D. Lockie and J. B. Sprowls, *ibid.*, **40**, 72(1951).

(10) C. W. Whitworth and C. H. Becker, *J. Pharm. Sci.*, **54**, 569(1965).

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NOTES

Synthetic Procedures for Deuterium-Labeled Acetylcholine Perchlorates

JOHN L. HOGG and R. L. SCHOWEN [x]

Abstract □ Synthetic procedures for selectively deuterated acetylcholine perchlorates are reported. Acetyl-d_3-choline perchlorate (I) was prepared *via* esterification of acetic acid-d_4 with ethylene bromohydrin followed by quaternization of the bromoester with trimethylamine and treatment with perchloric acid. N-$(CD_3)_3$-Acetylcholine perchlorate (II) was prepared by quaternization of ethanolamine with methyl-d_3 iodide, acetylation with acetic anhydride, and anion exchange using silver perchlorate. Lithium aluminum deuteride-d_4 reduction of ethyl bromoacetate gave 1,1-d_2-2-bromoethanol (VI), which was esterified with acetic acid followed by quaternization and anion exchange, as for I, to give acetylcholine-1,1-d_2 perchlorate (III). A similar sequence was used to prepare acetylcholine-2,2-d_2 perchlorate (IV) from 2,2-d_2-2-bromoethyl acetate (X).

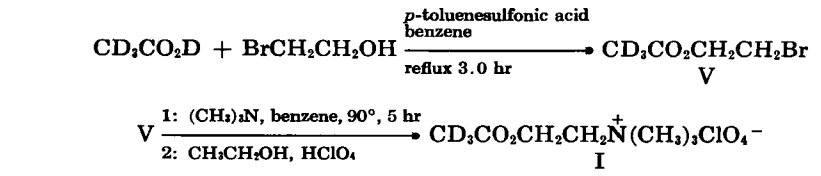
Keyphrases □ Acetylcholine perchlorates, deuterium labeled—synthesis □ Deuterium-labeled acetylcholine perchlorates—synthesis

The enzyme acetylcholinesterase catalyzes the hydrolysis of the natural substrate acetylcholine in carrying out its central role in the function of the nervous system (1). Studies in this laboratory required the syntheses of four specifically labeled acetylcholine salts: $CD_3CO_2CH_2CH_2N^+(CH_3)_3ClO_4^-$, I; $CH_3CO_2CH_2CH_2N^+(CD_3)_3ClO_4^-$, II; $CH_3CO_2CD_2CH_2N^+(CH_3)_3ClO_4^-$, III; and $CH_3CO_2CH_2CD_2N^+(CH_3)_3ClO_4^-$, IV.

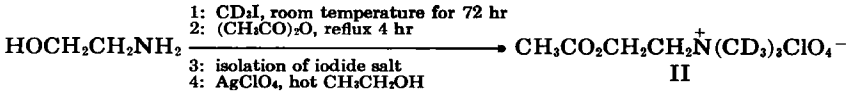
The syntheses of I and II, using reported procedures (2–4), are given in very limited detail (5) as are the syntheses of III and IV (5). Poor results were obtained in this group using many reported procedures, so modified procedures were developed and are reported here in detail. The compounds were isolated as their perchlorate salts since these have the distinct advantage of being nonhygroscopic as opposed to the halide salts (6). Compounds I and II could be obtained in relatively high yields, but the overall yields of III and IV could never be raised above a few percent due to the difficulties encountered in preparing the necessary labeled ethylene bromohydrins [$BrCH_2CD_2OH$ (VI) and $BrCD_2CH_2OH$ (IX)].

DISCUSSION

The two methods used for the preparation of the ethylene bromohydrins gave very low yields, even though the reactions were re-



Scheme I



Scheme II

peated many times taking every possible precaution. The compounds could not be isolated in a very pure state, and the yields of the subsequent esterification reactions were lower than model reactions would have predicted. The addition of aluminum chloride to the reduction mixture did appear to improve the yield of the reduction (7). The preparation of ethylene chlorohydrins in relatively high yields was reported (8) from the acid chlorides or ethyl esters, and these yields could be repeated almost exactly, which assured that no gross error was being made in experimental technique. This procedure might have offered a solution to the problems mentioned, except that the final quaternization gave yields of only 5–10%. Continuous extraction of the water phase from the workup of the reduction according to Nystrom (9) gave little improvement.

Methods for the preparation of Compounds I–IV are given in Schemes I–IV, respectively.

EXPERIMENTAL[1]

2-Bromoethyl Acetate-d_3 (V)—A reaction flask, fitted with a graduated 2-ml Dean-Starke trap, a microcondenser, and a calcium sulfate drying tube in series, was charged with 2.50 g (3.90×10^{-2} mole) of acetic acid-d_4 (99.5% minimum isotopic purity)[2]. To this was added 4.90 g (3.92×10^{-2} mole) of 2-bromoethanol dissolved in 2.5 ml of reagent grade benzene and 0.15 g (7×10^{-4} mole) of *p*-toluenesulfonic acid monohydrate. An additional 2.5 ml of benzene was used to fill the side arm of the trap to its maximum capacity. The reaction solution was heated at reflux, with magnetic stirring, for 3.0 hr, during which time 0.71 ml of water (3.9×10^{-2} mole) was collected. After cooling, the reaction solution was distilled at atmospheric pressure, with fractions distilling below 150° being discarded. The distillation afforded 4.66 g (70%) of V, bp 159–161° [lit. (10) bp 162–163° for the nondeuterated analog]; IR (CCl_4): 2975 and 1730 cm^{-1}; NMR (CCl_4): δ 2.05 (~0.1H, s)[3], 3.45 (2H, t), and 4.30 (2H, t).

Acetyl-d_3-choline Perchlorate (I)—According to the procedure of Fourneau and Page (3), a pressure bottle, precooled to −15° in an ice–salt bath, was charged with 4.50 g (2.64×10^{-2} mole) of V in 25.0 ml of dry benzene. To this was added 7.71 g (1.31×10^{-1} mole) of anhydrous trimethylamine, and the pressure bottle was sealed. The solution was allowed to warm to room temperature and was then heated, with magnetic stirring, in a silicone oil bath at 90° for 5 hr. The flask was then cooled to −15°, opened cautiously, and allowed to warm to room temperature gradually. Concentration of the reaction solution at reduced pressure gave approximately 5.1 g of slightly wet crystals, which were then dissolved in 38 ml of absolute ethanol and treated with 3.87 g (2.70×10^{-2} mole) of 70% aqueous perchloric acid with stirring and cooling in an ice bath. The resultant white, feathery crystals were stored in the mother liquor overnight in the refrigerator, collected *via* suction filtration, washed with cold ether, and dried *in vacuo* to give 4.67 g (71%) of I, mp 110.0–111.5°. Recrystallization from absolute ethanol gave 4.10 g, mp 113.5–114.5° [lit. (6) mp 116–117° for the nondeuterated analog]; IR (mineral oil): 2941, 2033, and 1733 cm^{-1}; NMR (D_2O): δ 3.60 (9H, s), 4.15 (2H, m), and 4.95 (2H, m).

Anal.[4]—Calc. for $C_7H_{13}D_3ClNO_6$: C, 33.81; "H," 6.49; Cl, 14.26; N, 5.63; atom-% excess D, 18.75. Found: C, 33.97; "H," 6.62; Cl, 13.92; N, 5.78; atom-% excess D, 17.55. These values convert to 93.7% deuteration at the desired site.

***N*-$(CD_3)_3$-Acetylcholine Perchlorate (II)**—According to the procedure of du Vigneaud *et al.* (4), a reaction flask was precooled to dry ice–acetone temperature and charged with 1.40 g (2.92×10^{-2} mole) of ethanolamine. To this was added 5.0 g (3.44×10^{-2} mole) of methyl-d_3 iodide (99.5 atom-% D)[5] and the flask was immediately topped with a condenser and calcium sulfate drying tube. The solution was then allowed to warm to room temperature, at which point it began to reflux spontaneously for about 10 min. After 72 hr of magnetic stirring at room temperature, the viscous orange reaction solution was treated with 7.18 g (7.02×10^{-2} mole) of acetic anhydride (11). The resultant mixture was heated at reflux for 4 hr, cooled to room temperature, and concentrated *in vacuo*. The resultant red oil was treated with 1.50 ml of absolute ethanol to give pale-yellow crystals. Isolation of these crystals followed by two successive recrystallizations from absolute ethanol gave 1.17 g of *N*-$(CD_3)_3$-acetylcholine iodide, which was slowly added to a hot solution of 0.87 g (4.2×10^{-3} mole) of silver perchlorate in 25 ml of absolute ethanol. The yellow silver iodide, which immediately precipitated, was removed by suction filtration of the hot ethanol solution. The clear supernatant solution was allowed to cool to give, after two recrystallizations from hot ethanol, 0.66 g (23%) of II, mp 113.5–114.5° [lit. (6) mp 116–117° for the nondeuterated analog]; NMR (D_2O): δ 2.45 (3H, s), 4.05 (2H, m), and 4.80 (2H, m).

Anal.—Calc. for $C_7H_9D_9ClNO_6$: C, 33.01; "H," 6.33; Cl, 13.92; N, 5.50; atom-% excess D, 56.25. Found: C, 33.41; "H," 6.78; Cl, 13.64; N, 5.50; atom-% excess D, 54.00. These values convert to 96.00% deuteration at the desired site.

1,1-d_2-2-Bromoethanol (VI)—An erlenmeyer flask was charged with 2.50 g (5.98×10^{-2} mole) of lithium aluminum deuteride-d_4 (99 atom-% D)[5] and 7.97 g (5.98×10^{-2} mole) of anhydrous aluminum chloride and stoppered. A three-necked flask was fitted with a pressure-equalizing addition funnel, a condenser and drying tube, and a 20.3-cm section of 2.54-cm diameter (0.3-cm walls) rubber tubing. The erlenmeyer flask containing the deuteride and chloride was fitted into the end of the tubing and clamped so as to bend the tubing shut. The reaction flask was then charged with 50 ml of rigorously dried ether (distilled over lithium aluminum hydride) using a syringe. The reaction flask was cooled in a dry ice–acetone bath, and the lithium aluminum deuteride–aluminum chloride mixture was cautiously added, with magnetic stirring of the ether solution, using the tubing as a solid addition device. The dry ice–acetone bath was removed and the slurry was treated with 10.0 g (5.98×10^{-2} mole) of ethyl bromoacetate over 15 min. The resultant mixture was then stirred at room temperature for 1 hr and treated with 27.5 ml of water followed by 27.5 ml of 6 *N* sulfuric acid. The aqueous phase was separated, saturated with sodi-

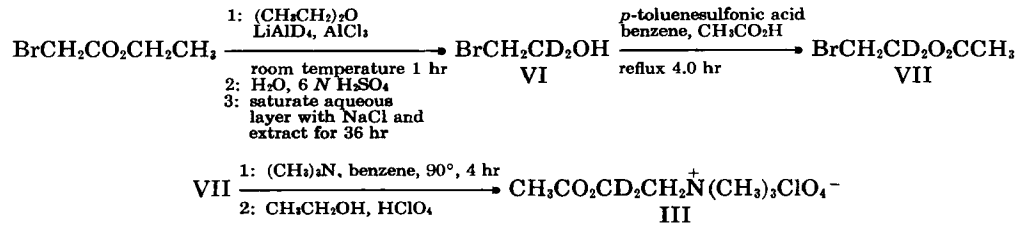
[1] IR spectra were taken on a Perkin-Elmer IR-8, NMR spectra were taken on a Varian T-60, and melting points were determined on a Thomas Hoover apparatus and are uncorrected. Elemental analyses were performed by Mr. Josef Nemeth.

[2] Diaprep.

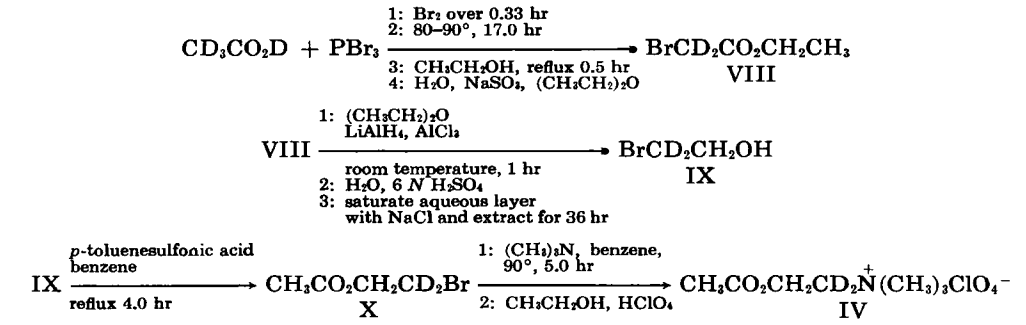
[3] Based on the assumption that the other two signals represented a total of 4H.

[4] The "H" means that the H and D were both considered together, since the gravimetric method used by Mr. Nemeth does not distinguish between H and D. The atom-% excess D values were used to calculate the percent deuteration of the samples.

[5] International Chemical and Nuclear Corp.



Scheme III



Scheme IV

um chloride, and continuously extracted with ether for 36 hr. The combined ether phases were dried over magnesium sulfate, filtered, concentrated, and distilled to give 4.73 g (62%) of VI[6], bp 65–68° (33 mm) [lit. (12) bp 55–59° (22 mm) for the nondeuterated analog].

1,1-d_2-2-Bromoethyl Acetate (VII)—According to the same procedure given for the preparation of V, 4.60 g (3.62×10^{-2} mole) of VI was reacted with 2.19 g (3.65×10^{-2} mole) of acetic acid and 0.1 g (5×10^{-4} mole) of *p*-toluenesulfonic acid monohydrate in benzene for 4.5 hr. Only about 90% of the theoretical amount of water was collected. Distillation as before gave 2.57 g (42%) of VII, bp 160–162° [lit. (10) bp 162–163° for the nondeuterated analog]; IR (CCl_4): 3012, 2160, 1742, and 1706 cm^{-1}; NMR (CCl_4): δ 2.10 (3H, s), 3.50 (1H, s), and 4.35 (1H, s).

Acetylcholine-1,1-d_2 Perchlorate (III)—According to the procedure described for the preparation of I, 1.10 g (6.50×10^{-3} mole) of VII and 6.71 g (1.13×10^{-1} mole) of anhydrous trimethylamine were heated in 20 ml of benzene for 4 hr. Treatment of the resultant concentrated solution in 15 ml of absolute ethanol with 0.75 ml of 70% aqueous perchloric acid allowed isolation of the compound as feathery, white crystals. Successive recrystallizations from ethanol yielded 0.1308 g (8%) of III, mp 111–112° [lit. (6) mp 116–117° for the nondeuterated analog].

Anal.—Calc. for $C_7H_{14}D_2ClNO_6$: C, 33.94; "H," 5.69; Cl, 14.31; N, 5.65; atom-% excess D, 12.50. Found: C, 34.42 and 34.41; "H," 6.47 and 6.51; Cl, 14.33; N, 5.84; atom-% excess D, 12.10. This converts to 99.6% deuteration at the desired site.

Ethyl Bromoacetate-d_2 (VIII)—A reaction flask was charged with 5.00 g (7.80×10^{-2} mole) of acetic acid-d_4 (99.5 atom-% D)[5] and 21.2 g (7.81×10^{-2} mole) of phosphorus tribromide to give a colorless cloudy solution (13). To this was added, over 20 min, 31.20 g (1.90×10^{-1} mole) of bromine. This gave an initial cloudy yellow solution, which began to reflux spontaneously about 5 min into the addition. The resultant blood-red solution was heated at 80–90° for 17 hr. The reaction solution was then treated with 50 ml of absolute ethanol while cooling in an ice bath and heated for 30 min more. The solution was then cooled to room temperature and treated with about 100 ml of water containing enough sodium sulfite to give a colorless solution. This was then extracted with ether, with the ether phases being dried over sodium sulfate. Concentration and distillation gave 6.28 g (48%) of VIII, bp 154–156° [lit. (14) bp 159° for the nondeuterated analog]; NMR ($CDCl_3$): δ 1.35 (3H, t) and 4.30 (2H, q).

2,2-d_2-2-Bromoethanol (IX)—According to the same procedure given for the preparation of VI, 6.10 g (3.60×10^{-2} mole) of VIII, 1.37 g (3.60×10^{-2} mole) of lithium aluminum hydride, and 4.80 g (3.60×10^{-2} mole) of anhydrous aluminum chloride were reacted to give 1.95 g (43%) of IX[7], bp 63.0–64.5° (30 mm) [lit. (12) bp 56–57° (20 mm) for the nondeuterated analog].

2,2-d_2-2-Bromoethyl Acetate (X)—According to the procedure reported for the preparation of VII, 1.70 g (1.34×10^{-2} mole) of IX was allowed to react with 0.81 g (1.35×10^{-2} mole) of acetic acid. Compound X was collected in a yield of 1.04 g (46%), bp 157–159° [lit. (10) bp 161.5° for the nondeuterated analog]; IR (CCl_4): 3497, 2941, 2169, and 1742 cm^{-1}; NMR (CCl_4) δ 1.65 (3H, s), 3.25 (1H, s), and 3.70 (1H, s).

Acetylcholine-2,2-d_2 Perchlorate (IV)—According to the procedure described for the preparation of I, 1.0 g (5.93×10^{-3} mole) of X and 6.71 g (1.13×10^{-1} mole) of anhydrous trimethylamine were heated for 5 hr in 20 ml of benzene. Treatment of the resultant concentrated solution with 70% aqueous perchloric acid as before and recrystallization of the resultant white, feathery crystals gave 0.50 g (34%) of IV, mp 113.5–114.5° [lit. (6) mp 116–117° for the nondeuterated analog].

Anal.—Calc. for $C_7H_{14}D_2ClNO_6$: C, 33.95; "H," 6.50; Cl, 14.31; N, 5.66; atom-% excess D, 12.50. Found: C, 33.80; "H," 6.39; Cl, 14.17; N, 5.62; atom-% excess D, 12.45. This converts to 99.6% deuteration at the desired site.

REFERENCES

(1) H. C. Froede and I. B. Wilson, in "The Enzymes," vol. V, 3rd ed., P. D. Boyer, Ed., Academic, New York, N.Y., 1971, chap. 5.

(2) H. Erlenmeyer and H. Lobeck, *Helv. Chim. Acta*, **20,** 142(1937).

(3) E. Fourneau and H. J. Page, *Bull. Soc. Chim. Fr.*, **15,** 544(1914).

(4) V. du Vigneaud, M. Cohn, J. P. Chandler, J. R. Schenck, and S. Simmonds, *J. Biol. Chem.*, **140,** 625(1941).

(5) B. Belleau, in "Isotopes in Experimental Pharmacology," L. J. Roth, Ed., University of Chicago Press, Chicago, Ill., 1965, chap. 36.

(6) F. K. Bell and C. J. Carr, *J. Amer. Pharm. Ass., Sci. Ed.*, **36,** 272(1947).

(7) E. L. Eliel and M. N. Rerick, *J. Amer. Chem. Soc.*, **82,** 1362(1960).

(8) C. E. Sroog, C. M. Chih, F. A. Short, and H. M. Woodburn, *ibid.*, **81,** 610(1959).

(9) R. F. Nystrom, *ibid.* **81,** 610(1959).

(10) E. Demole, *Ann.*, **173,** 121.

[6] This compound was also prepared in 38% yield by lithium aluminum deuteride reduction of bromoacetyl bromide.

[7] This compound was also prepared in 35% yield by lithium aluminum hydride reduction of deuterated bromoacetyl bromide which had been prepared by the action of bromine and red phosphorus on acetic acid-d_4.

(11) D. E. Walz, M. Fields, and J. A. Gibbs, *J. Amer. Chem. Soc.*, **73**, 2968(1951).
(12) K. F. Thayer, C. S. Marvel, and G. S. Hiers, *Org. Syn.*, **6**, 12(1926).
(13) C. F. Allen and M. J. Kalm, *Org. Syn., Coll. Vol.*, **4**, 608(1963).
(14) W. H. Perkins and B. F. Duppa, *Ann.*, **108**, 110(1858).

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[x] To whom inquiries should be directed.

Euparone, a New Benzofuran from *Ruscus aculeatus* L.

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Abstract □ The structure of euparone, a new benzofuran from *Ruscus aculeatus*, as 2,5-diacetyl-6-hydroxybenzofuran was determined by spectral means and conversion to euparone methyl ether (*O*-methyleuparone).

Keyphrases □ *Ruscus aculeatus* L. constituents—structure determination of euparone □ Euparone (2,5-diacetyl-6-hydroxybenzofuran)—structure determination □ 2,5-Diacetyl-6-hydroxybenzofuran—structure determination □ Benzofurans—from *R. aculeatus*, structure determination

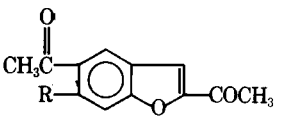
Ruscus aculeatus (Liliaceae), also known as Butcher's Broom or Knee-Holly, is a low evergreen shrub of southern and western Europe and southern United States (1). It has been used medicinally as a diuretic (2) and an anti-inflammatory (3) agent, as well as to treat hemorrhoids (4) and to prevent atherosclerosis (5) and circulatory insufficiency (6). Extracts of various *Ruscus* species have been shown to contain saponins (7) and flavonoids (8). This paper reports the isolation and structure determination of euparone, a new benzofuran.

DISCUSSION

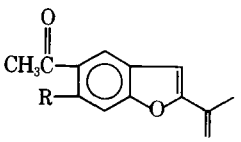
Euparone (I) was isolated as yellow rhomboid crystals from an ethanolic extract of the roots of *R. aculeatus*. Some pertinent data are: $C_{12}H_{10}O_4$; mp 143° ($CHCl_3$–C_2H_5OH); $[\alpha]_D$ 0° (c 1.0, $CHCl_3$); $\lambda_{max}(CH_3OH)$: 228 (sh) (log ϵ 4.19), 233 (4.21), 254 (4.37), 266 (sh) (4.35), 303 (4.11), and 348 (4.07) nm; λ_{max}(0.1 *N* methanolic KOH): 210 (log ϵ 4.32), 244 (4.00), 260 (sh) (3.82), 291 (3.66), 343 (3.78), and 403 (4.01) nm; ν_{max}(KBr): 3150 (bonded OH), 1667 (CO), and 1635 (CO) cm^{-1}; $\delta_{60\ MHz}(CDCl_3)$: 2.57 (s, 3H, $COCH_3$), 2.70 (s, 3H, $COCH_3$), 7.10 (s, 1H, Ar), 7.50 (s, 1H, Ar), 8.23 (s, 1H, Ar), and 12.66 (s, 1H, OH); mass spectroscopy M^+: *m/e* 218 (59%), 203 (100), and 43 (18). The spectral data were indicative of a phenolic 2,5,6-trisubstituted benzofuran containing two acetyl groups, one of which (1635 cm^{-1}) was *ortho* to a phenolic hydroxy (δ 12.66) group (9–12). Euparone gave a positive iodoform reaction characteristic of a methyl ketone. Treatment of euparone with acetic anhydride and pyridine afforded a monoacetate (II), $C_{14}H_{12}O_5$; mp 126° ($CHCl_3$–C_2H_5OH); $\lambda_{max}(CH_3OH)$: 227 (sh) (log ϵ 3.97), 249 (4.20), 287 (3.98), and 320 (sh) (3.68) nm; ν_{max}(KBr): 1765 (ArO-$COCH_3$) and 1665 (CO) cm^{-1}; $\delta_{60MHz}(CDCl_3)$: 2.36 (s, 3H, $COCH_3$), 2.58 (s, 6H, $2COCH_3$), 7.31 (s, 1H, Ar), 7.50 (s, 1H, Ar), and 8.15 (s, 1H, Ar); mass spectroscopy M^+: *m/e* 260 (2%), 218 (88), 203 (100), and 43 (30).

A further indication of the bonded nature of the hydroxy group of euparone was the failure of the compound to form a methyl ether on treatment with diazomethane. However, treatment of euparone with methyl iodide and silver oxide in refluxing chloroform afforded *O*-methyleuparone (euparone methyl ether) (III), $C_{13}H_{12}O_4$; mp 135–136° ($CHCl_3$–CH_3OH); $\lambda_{max}(CH_3OH)$: 232 (log ϵ 4.11), 245 (sh) (4.16), 251 (4.19), 299 (4.07), and 330 (4.11) nm; ν_{max}(KBr): 1685 (CO) and 1665 (CO) cm^{-1}; $\delta_{60\ MHz}(CDCl_3)$: 2.56 (s, 3H, $COCH_3$), 2.63 (s, 3H, $COCH_3$), 3.97 (s, 3H, OCH_3), 7.09 (s, 1H, Ar), 7.46 (s, 1H, Ar), and 8.05 (s, 1H, Ar); mass spectroscopy M^+: *m/e* 232 (48%), 217 (100), 202 (14), and 43 (28). This compound was identical with an authentic sample of euparone methyl ether by direct comparison (UV, IR, NMR, mass spectroscopy, melting point, and mixed melting point).

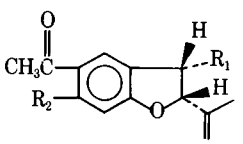
To the knowledge of the authors, this is the first reported isolation of euparone from nature. Euparone methyl ether (*O*-methyleuparone) (III), the first 2-acetylbenzofuran of nature, was first isolated from *Encelia californica* (Compositae) and was so named because of the obvious structural relationship to its probable 2-isopropenyl precursor, euparin (IV), also present in *E. californica* (13). Other 2,5-disubstituted benzofurans of nature include 2,5-diacetylbenzofuran (V) from *Aplopappus* species (14); tremetone (VI), dehydrotremetone (VII), and hydroxytremetone (VIII) (as a group formerly called tremetol) from *Eupatorium* (15, 16) and *Ap-*



I: R = OH
II: R = $OCOCH_3$
III: R = OCH_3
V: R = H



IV: R = OH
VII: R = H



VI: $R_1 = R_2 = H$
VIII: $R_1 = H, R_2 = OH$
IX: $R_1 = OH, R_2 = H$