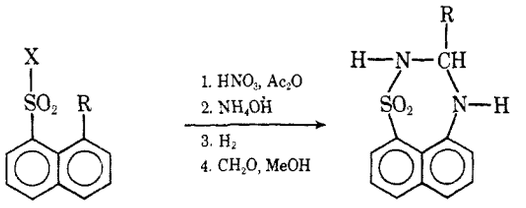
In method B (Scheme II) the intermediate VIII was obtained (as reported by Joy and Bogert[3]) and ammoniated to compound IX which was then reduced with

SCHEME II



VII, X = Cl; R = H
VIII, X = Cl; R = NO_2
IX, X = NH_2; R = NO_2
X, X = NH_2; R = NH_2

XIa, R = H
b, R = $C_6H_5CH_2$

hydrogen in the presence of Raney nickel catalyst. Compound IX was identical with that reported by Heller.[4] The ring closure was performed as described in method A.

Experimental Section

Pyridinium 3-(N-Acetylanilino)-1-naphthalenesulfonate (II).—8-Anilino-1-naphthalenesulfonic acid (I) (100 g, 0.31 mol) was suspended in 200 ml of pyridine and refluxed in 400 ml of acetic anhydride for 2 hr. The crystals were filtered and washed with acetone: mp 217–219°; yield 87 g (63%).

Anal. Calcd for $C_{23}H_{20}N_2O_4S$: C, 67.29; H, 4.91; N, 6.82. Found: C, 67.32; H, 4.88; N, 6.89.

8-(N-Acetylanilino)-1-naphthalenesulfonyl Chloride (III).—Pyridinium 8-(N-acetylanilino)-1-naphthalenesulfonate (II) (42 g, 0.09 mol) and 21 g (0.1 mol) of PCl_5 wih 50 ml of PCl_3 were mixed and refluxed for 5 min. The syrupy mass was poured into ice with good stirring, and the formed crystals, after recrystallizing from benzene, had mp 140–141°; yield 23.0 g (71%).

Anal. Calcd for $C_{18}H_{14}NO_3SCl$: C, 60.08; H, 3.92; N, 3.90; S, 8.92; Cl, 9.86. Found: 59.92; H, 3.87; N, 3.92; S, 8.81; Cl, 9.92.

8-N-Acetylanilino-1-naphthalenesulfonamide (IV).—8-(N-acetylanilino)-1-naphthalenesulfonyl chloride (III) (23 g, 0.064 mol) was boiled with a mixture of 300 ml of 10% ammonia solution and 200 ml of methanol for 2 hr and allowed to stand for 2 hr at room temperature. The fine crystals were then filtered off and washed with 150 ml of water and twice with 50 ml of methanol. After recrystallizing from 50% methanol, the product had mp 212–214°; yield 21.0 g (96%).

Anal. Calcd for $C_{18}H_{16}N_2O_3S$: C, 63.49; H, 4.74; N, 8.24 S, 9.24. Found: C, 63.25; H, 4.80; N, 8.23; S, 8.70.

8-Aniliño-1-naphthalenesulfonamide (V).—8-(N-Acetylanilino)-1-naphthalenesulfonamide (IV) (21 g, 0.062 mol) was hydrolyzed with 350 ml of 5% NaOH solution for 17 hr. The mixture was filtered, and to the filtrate 75 ml of 50% NH_4Cl solution was added. The formed crystals were collected, washed twice with 25 ml of water, and recrystallized from MeOH with the aid of a charcoal decolorizing agent. The yield was 13 g (67%).

Anal. Calcd for $C_{16}H_{14}N_2O_2S$: C, 64.63; H, 4.41; N, 9.42; S, 10.78. Found: C, 64.43; H, 4.43; N, 9.28; S, 11.00.

Naphthalene[1,8-*e,f*]-2,3-dihydro-4H-4-phenyl-[1.2.4]thiadiazepine 1,1-Dioxide (VI).—A solution of 5 g (0.0155 mol) of 8-anilino-1-naphthalenesulfonamide (V) 70 ml of absolute MeOH and 2 ml of 55% Methyl Formcel was refluxed for 8 min. The mixture was cooled and allowed to stand at room temperature overnight. The formed crystals yielded 2.5 g (48%); the product had mp 178°.

Anal. Calcd for $C_{17}H_{14}N_2O_2S$: C, 65.78; H, 4.54; N, 9.02; S, 10.33. Found: C, 65.51; H, 4.27; N, 8.47; S, 10.15.

(3) H. V. Joy and M. T. Bogert, *J. Org. Chem.*, **1**, 236 (1936).
(4) J. Heller, *J. Prakt. Chem.*, **2**, 121, (1940).

8-Nitro-1-naphthalenesulfonyl chloride (VIII).—This compound was prepared by the method of Joy and Bogert:[3] yield 17%; mp 153–156° (lit.[3] mp 161–162°).

8-Nitro-1-naphthalenesulfonamide (IX).—This was prepared from compound VIII by boiling the latter in methanolic ammonia: yield 95%; mp 188–190° (lit.[5] mp 190.5–1.5°).

8-Amino-1-naphthalenesulfonamide (X).—The compound IX (1.5 g, 0.0060 mol) was reduced catalytically over Raney nickel in ethanol. The solvent was removed, giving a crystalline product which was then recrystallized from 3 ml of benzene–methanol–petroleum ether (bp 40–60°): yield 1.0 g (75%); mp 189–191°.

Anal. Calcd for $C_{10}H_{10}N_2O_2S$: C, 54.45; H, 4.53; N, 12.60; S, 14.42. Found: C, 54.41; H, 4.56; N, 12.62; S, 14.38.

Naphthalene-[1,8-*e,f*]-2,3-dihydro-4-H-[1,2,4]-thiadiazepine 1,1-Dioxide (XIa).—A solution of 958 mg (0.0044 mol) of 8-amino-1-naphthalenesulfonamide (X) in 450 ml of MeOH was refluxed with 15 drops of Methyl Formcel[2] (55%) for 10 min. The reaction mixture was then reduced to a 70-ml volume, and, on cooling, crystals formed which were collected and recrystallized from 4 ml of benzene: yield 750 mg (75%); mp 214–216°.

Anal. Calcd for $C_{11}H_{10}N_2O_2S$: C, 56.39; H, 4.30; N, 11.95; S, 13.68. Found: C, 56.23; H, 4.46; N, 11.83; S, 13.75.

3-Benzylnaphthalene-[1,8-*e,f*]-2,3-dihydro-4-H-[1,2,4]-thiadiazepine 1,1-Dioxide (XIb).—To a solution of 2.0 g (0.0090 mol) of 8-amino-1-naphthalenesulfonamide (X) in 50 ml of ethanol was added 4 ml of phenylacetaldehyde in ethanol (50%). The mixture was refluxed for 1.5 hr. This mixture was concentrated to one-half volume, and, on cooling, crystals formed which were collected, washed twice with 1 ml of methanol and petroleum ether, and finally recrystallized from 130 ml of methanol: yield 1.5 g (71%); mp 176–176.5°.

Anal. Calcd for $C_{18}H_{16}N_2O_2S$: C, 66.64; H, 4.97; N, 8.63; S, 9.88. Found: C, 66.25; H, 5.06; N, 8.83; S, 10.15.

Registry No.—II, 16888-87-2; III, 16888-81-6; IV, 16888-82-7; V, 16888-83-8; VI, 16932-58-4; X, 16888-84-9; XIa, 16888-85-0; XIb, 16888-86-1.

Acknowledgment.—Thanks are due to G. Robertson, Jr., Florham Park, N. J., for the microanalyses.

(5) R. E. Steiger, *Helv. Chim. Acta*, **17**, 701 (1934).

The Isolation and Structural Elucidation of 4-Demethylhasubanonine, a New Alkaloid from *Stephania hernandifolia*

S. MORRIS KUPCHAN,[1a] MATTHEW I. SUFFNESS,[1a] D. N. J. WHITE,[1b] A. T. MCPHAIL,[1b] AND G. A. SIM[1b]

Department of Pharmaceutical Chemistry, University of Wisconsin, Madison, Wisconsin 53706 and Chemical Laboratory, University of Sussex, Brighton, Sussex, England

Received July 8, 1968

Stephania hernandifolia Walp. is a menispermaceous slender twining shrub found in India on the west and east coasts, in Cachar, Sikkim, East Bengal, and Assam.[2] The roots are reported to have use in the treatment of fever, diarrhea, dyspepsia, and urinary diseases.[3]

(1) (a) University of Wisconsin. The investigation at the University of Wisconsin was supported by Public Health Service Grant No. HE-02952 from the National Heart Institute. M. I. S. was an National Institutes of Health Predoctoral Fellow, 1966–1968. (b) University of Sussex.

(2) R. N. Chopra, L. C. Chopra, K. L. Handa, and L. D. Kapur, "Indigenous Drugs of India," 2nd ed, U. N. Dhur and Sons, Calcutta, 1958.

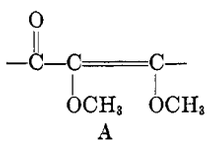
(3) R. N. Chopra, S. L. Nayar, and I. C. Chopra, "Glossary of Indian Medicinal Plants," Council of Scientific and Industrial Research, New Delhi, 1956.

An examination of *S. hernandifolia* from the eastern coast of Australia revealed the presence of *l*-quercitol and a mixture of alkaloids.[4] In 1959,[5] Tomita and Ueda reported on the alkaloids of *S. hernandifolia* purchased as crude drug on the Bombay market. They isolated the alkaloid isotrilobine, a new tertiary phenolic base, and β-sitosterol. Moza, *et al.*, have reported that the plant contained alkaloids, steroids, and fats,[6a] and have partially characterized three alkaloids.[6b] A recent communication has described the partial characterization of an alkaloid of empirical formula $C_{20}H_{25}NO_5$.[7]

In an earlier report from this laboratory, the isolation from *S. hernandifolia* from India of the alkaloids *dl*-tetrandrine, fangchinoline, *d*-tetrandrine, and *d*-isochondrodendrine was reported.[8] Subsequent biological studies demonstrated that all four alkaloids showed significant cytotoxicity against human carcinoma of the nasopharynx carried in tissue culture (KB),[9] and that *dl*-tetrandrine and *d*-tetrandrine showed significant inhibitory activity *in vivo* against the Walker 256 intramuscular carcinosarcoma in the rat.

The present communication concerns an investigation of a new sample of roots of *S. hernandifolia*, collected in India in Jan 1965.[10] Careful examination of the alkaloid mixture revealed that this plant sample contained none of the alkaloids found earlier. We report herein the isolation and structural elucidation of 4-demethylhasubanonine (Ia), a new phenolic alkaloid of the hasubanan series.[11]

A concentrated methanolic extract of the defatted roots of *S. hernandifolia* Walp. was triturated with 6% hydrochloric acid, and the acid solution was basified with ammonium hydroxide and extracted with chloroform to yield the crude nonquaternary alkaloids.[12] The crude alkaloids were fractionated by continuous ether extraction. The ether-soluble alkaloids were chromatographed on silica to yield a fraction rich in the new alkaloid. Treatment with oxalic acid in methanol and repeated recrystallizations from methanol–ether yielded 4-demethylhasubanonine (Ia) oxalate salt, $C_{20}H_{25}NO_5 \cdot C_2H_2O_4$: mp 198–199°; $[\alpha]^{32}D$ −123°; *m/e* 359;[13] λ_{max}^{EtOH} 266 mμ (ε 8400). The infrared spectrum (KBr) showed absorption at 5.95 μ, indicative of α,β-unsaturated ketone. The alkaloid was also characterized as the perchlorate salt (mp 229–230°; $[\alpha]^{32}D$ −145°) and the brosylate ester (Ib) (mp 209–211°; $[\alpha]^{29}D$ −149°, M^+ *m/e* 576). The amorphous free base showed nmr signals at τ 3.32 and 3.45 (2 H, q, J = 9 cps, 2 aromatic H), 3.88 (1 H, OH), 5.92, 6.18, and 6.32 (9 H, 3 OCH_3), 7.48 (3 H, NCH_3). The infrared and nmr spectra indicated the presence of the partial structure A. Characterization of the



A

hydroxyl group as a phenolic hydroxyl group with an unsubstituted *para* position was supported by the positive reaction toward Gibbs reagent.

Methylation of the alkaloid with diazomethane gave hasubanonine (Ic), characterized by comparison of the physical constants of the alkaloid and its methiodide derivative with those reported in the literature.[14,15] The foregoing data established the demethylhasubanonine nature of the new alkaloid.

A comparison of the nmr signals for the methoxyl groups of the new alkaloid with those of hasubanonine (Ic), cepharamine (Id), homostephanoline (IIa), and O-ethylhomostephanoline (IIb)[16,17] (see Table I) limited alternative assignments of the free hydroxyl group to C-3 or C-4. Since the alkaloid was different from the C-3 phenolic isomer, homostephanoline (IIa), the 4-demethylhasubanonine structure (Ia) appeared most plausible. Structure Ia was supported also by the fact that the alkaloid reacted positively toward Gibbs reagent.

TABLE I

NMR METHOXYL RESONANCES IN THE HASUBANAN SERIES[a]

Compound	C-3	C-4	C-7	C-8
Ia	6.18		6.32	5.92
Ic	6.19	6.09	6.36	5.92
Id	6.15		6.35	
IIa		6.14	6.40	5.92
IIb		6.05	6.35	5.93

[a] All values are in τ for $CDCl_3$ solutions.

Unequivocal proof of structure Ia was achieved by X-ray crystallographic analysis of the brosylate Ib. The brosylate crystallized in the orthorhombic system, space group $P2_12_12_1$, with four molecules of $C_{26}H_{28}NO_7SBr$ in a cell of dimensions a = 8.52 Å, b = 24.23 Å, c = 12.60 Å. The X-ray diffraction data were recorded on equiinclination Weissenberg photographs, and visual estimation of the intensities gave a total of 1878 independent $|F_0|$ values.

Preliminary coordinates of the bromine and sulfur atoms were obtained from a Patterson synthesis, and the carbon, nitrogen, and oxygen atoms were then located in three-dimensional electron density distributions calculated with weighted Fourier coefficients.[18] Two Fourier syntheses completed the elucidation of the

(4) J. Ewing, G. K. Hughes, and E. Ritchie, *Aust. J. Sci. Res.*, **3A**, 514 (1950).

(5) M. Tomita and S. Ueda, *J. Pharm. Soc. Jap.*, **79**, 977 (1959).

(6) (a) B. K. Moza, *Indian J. Pharm.*, **22**, 63 (1960); (b) B. K. Moza and D. K. Basu, *ibid.*, **28**, 338 (1966).

(7) I. I. Fadeeva, A. D. Kuzovkov, and T. N. Il'inskaya, *Khim. Prir. Soedin.*, **3**, 106 (1967); *Chem. Abstr.*, **67**, 43966 (1967).

(8) S. M. Kupchan, W. L. Asbun, and B. S. Thyagarajan, *J. Pharm. Sci.*, **50**, 819 (1961).

(9) S. M. Kupchan, A. C. Patel, and E. Fujita, *ibid.*, **54**, 580 (1965).

(10) The authors acknowledge with thanks the receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture, Beltsville, Md., in accordance with the program developed with the U. S. Department of Agriculture by the Cancer Chemotherapy National Service Center (CCNSC), National Cancer Institute, National Institutes of Health.

(11) 4-Demethylhasubanonine is the sixth hasubanan alkaloid; *cf.* T. Ibuka and M. Kitano, *Chem. Pharm. Bull.* (Tokyo), **15**, 1944 (1967).

(12) We thank Riker Laboratories for the large-scale preparation of the alkaloidal extract, and the Cancer Chemotherapy National Service Center for arranging for the extraction, under Contract SA-43-PH-3764.

(13) We thank Dr. R. D. Brown and Dr. F. W. McLafferty of the Purdue Mass Spectrometry Center, supported under U. S. Public Health Service Grant FR-00354, for the mass spectral data.

(14) M. Tomita, T. Ibuka, Y. Inubushi, Y. Watanabe, and M. Matsui, *Chem. Pharm. Bull.* (Tokyo), **13**, 538 (1965).

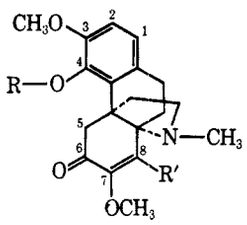
(15) M. Tomita, A. Kato, and T. Ibuka, *Tetrahedron Lett.*, 1019 (1965).

(16) T. Ibuka and M. Kitano, *Chem. Pharm. Bull.* (Tokyo), **15**, 1939 (1967).

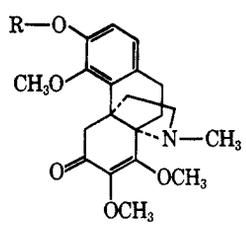
(17) M. Tomita and M. Kozuka, *Tetrahedron Lett.*, 6229 (1966).

(18) G. A. Sim in "Computing Methods and the Phase Problem in X-Ray Crystal Analysis," R. Pepinsky, J. M. Robertson, and J. C. Speakman, Ed., Pergamon Press, Oxford, 1961, p 227.

molecular structure as Ib, and an additional synthesis gave improved atomic coordinates. At this stage we included anomalous dispersion corrections in the structure factor calculations, and with coordinates appropriate to the absolute configuration shown in Ib the value of R was 19.1% whereas when the opposite configuration was tested R was 19.3%. These results are consistent with the assigned absolute configuration of hasubanonine.[19]



Ia, R = H; R′ = OCH_3
b, R = $SO_2C_6H_4Br$; R′ = OCH_3
c, R = CH_3; R′ = OCH_3
d, R = R′ = H



IIa, R = H
b, R = C_2H_5

Experimental Section

Melting points were determined with a Thomas–Hoover Unimelt apparatus and are corrected. Ir spectra were determined on Beckman double-beam recording spectrophotometers, Models IR-5A and IR-9. Uv spectra were determined in 95% ethanol on a Beckman recording spectrophotometer, Model DK2A. Optical rotations were measured with a Zeiss–Winkel polarimeter and are approximated to the nearest degree. Nmr spectra were determined on a Varian Associates spectrometer, Model A-60A. Skellysolve B is that fraction of petroleum ether boiling from 60 to 68°.

Extraction and Preliminary Fractionation.[12]—The dried root (41.4 kg) of *S. hernandifolia* was extracted twice with hexane (4 l./kg) and the hexane extracts were discarded. The defatted root was then extracted three times with methanol (3 l./kg) and twice with 70% methanol (3 l./kg) and the methanolic extracts were combined and concentrated *in vacuo* to a volume of 8 l. This material was triturated five times with 6% HCl (total of 200 l.) to give an acid solution and 1.3 kg of residual gums. The acid solution was extracted with ether (58 l.) to give 16.5 g of ether-extractable solids. The remaining acid layer was basified to pH 9.1 with NH_4OH and extracted twice with 80 l. of chloroform. The chloroform-soluble material yielded on evaporation the nonquaternary alkaloids (486 g). The aqueous solution was acidified to pH 1.8 with HCl and treated with ammonium Reineckate to precipitate the quaternary alkaloids (658 g, as Reineckates) and the remaining aqueous solution was then discarded. Continuous ether extraction of a portion of the nonquaternary alkaloids (172 g) for 21 days gave 96 g of ether-soluble alkaloids and left a residue of 75.5 g of ether-insoluble alkaloids.

4-Demethylhasubanonine (Ia).—The ether-soluble alkaloids (96 g) were chromatographed over 3.4 kg of SilicAR CC-7 (100–200 mesh, Mallinckrodt) in chloroform. After elution with chloroform (7 l.), the solvent was changed to 1% methanol–chloroform and after eluting with 4 l. of this solvent (*ca.* one retention volume) the crude 4-demethylhasubanonine was eluted with the next 9 l. of solvent. Fractions containing this material were combined to give 18.96 g of crude material which was dissolved in methanol (30 ml) and treated with 6.7 g of oxalic acid dihydrate in 30 ml of methanol. The resulting solution was heated to boiling on the steam bath, anhydrous ether was added to turbidity, and the mixture was allowed to crystallize. Two subsequent recrystallizations from methanol–ether gave pure 4-demethylhasubanonine (Ia) oxalate (12.32 g): mp 198–199°; $[\alpha]^{32}D$ −123° (*c* 3.46, methanol); λ_{max}^{EtOH} 266 mμ (ε 8400); λ_{max}^{KBr} 2.90, 3.39, 4.02, 5.85, 5.96 μ; *m/e* 359, 344, 328, 301, 300, 245.

Anal. Calcd for $C_{20}H_{25}NO_5 \cdot C_2H_2O_4$: C, 58.79; H, 6.06; N, 3.12. Found: C, 58.91; H, 6.02; N, 3.58.

4-Demethylhasubanonine Perchlorate.—4-Demethylhasubanonine oxalate (60 mg) was treated with excess 5% aqueous K_2CO_3 and extracted three times with chloroform (30 ml). The chloroform extracts were combined, dried (Na_2SO_4), and evaporated *in vacuo* to yield 48 mg of 4-demethylhasubanonine. A dilute methanolic solution of perchloric acid was added until the resulting solution was distinctly acid to litmus. The resulting solution was evaporated and the residue crystallized on addition of water. The crystalline material was filtered and recrystallized twice from chloroform–ether to give colorless needles (37 mg): mp 229–230°; $[\alpha]^{32}D$ −145° (*c* 0.51, methanol); λ_{max}^{EtOH} 262 mμ (ε 8360); λ_{max}^{KBr} 2.95, 3.25, 3.36, 5.95, 6.15, 6.69 μ.

4-Demethylhasubanonine Brosylate (Ib).—To *p*-bromobenzenesulfonyl chloride (2.71 g) in pyridine (dried over KOH) was added 236 mg of 4-demethylhasubanonine (liberated from oxalate salt as above) and the mixture was allowed to stand for 15 hr. The reaction mixture was taken up in 50 ml of chloroform and shaken with a saturated solution of $NaHCO_3$ to pH 8. The chloroform layer was removed and the aqueous phase extracted with three more portions of chloroform. The combined, dried (Na_2SO_4) chloroform solution was evaporated and residual pyridine was azeotroped with dry benzene to give a pale yellow oil which crystallized on trituration with Skellysolve B. Three crystallizations from acetone–Skellysolve B gave colorless needles (57 mg): mp 209–211°; $[\alpha]^{29}D$ −149° (*c* 0.42, $CHCl_3$); λ_{max}^{EtOH} 233 mμ (ε 21,750), 269 (14,650); λ_{max}^{Nujol} 3.41, 6.00, 6.21, 6.72, 7.29, 8.50 μ; *m/e* 578, 576, 358, 301, 300, 299, 158, 156.

Anal. Calcd for $C_{26}H_{28}NSO_7Br$: C, 53.97; H, 4.88; N, 2.42; Br, 13.82. Found: C, 54.06; H, 4.94; N, 2.39; Br, 13.72.

Hasubanonine (Ic).—A solution of diazomethane prepared from Diazald (4 g, Aldrich) was added to 280 mg of 4-demethylhasubanonine in methanol (5 ml). The reaction was kept in the dark at room temperature overnight. The resulting colorless solution was evaporated, dissolved in benzene, and chromatographed over 20 g of acid-washed alumina (Merck) in benzene. Elution was carried out with 25% chloroform–benzene and the fractions were monitored by tlc (5% methanol–chloroform on silica plates visualized by spraying with Dragendorff reagent) until no more product was eluted. The combined fraction from 25% chloroform–benzene gave pure hasubanonine (Ic, 162 mg). Subsequent elution with chloroform gave 125 mg of unreacted starting material. Oxalic acid dihydrate (54 mg) in methanol was added to a methanolic solution of the hasubanonine. Two recrystallizations of the product from methanol–ether gave colorless needles (93 mg): mp 192–193°; $[\alpha]^{32}D$ −134° (*c* 0.82, methanol); λ_{max}^{EtOH} 267 mμ (ε 8300); λ_{max}^{KBr} 2.90, 3.38, 4.08, 5.78, 5.94, 6.17, 6.71 μ.

Anal. Calcd for $C_{21}H_{27}NO_5 \cdot C_2H_2O_4$: C, 59.60; H, 6.31; N, 3.02. Found: C, 59.45; H, 6.51; N, 2.98.

Hasubanonine Hydrochloride.—Hasubanonine (55 mg) in chloroform (20 ml) was treated with HCl gas bubbled through the solution for 2 min. The solution was evaporated and the residue crystallized twice from chloroform–ether to give colorless needles (42 mg): mp 210–211°; $[\alpha]^{28}D$ −133° (*c* 1.14, methanol); λ_{max}^{EtOH} 268 mμ (ε 7300); λ_{max}^{Nujol} 3.41, 4.36, 5.96, 6.15, 6.72 μ; *m/e* 373, 358, 342, 315, 314, 284, 258, 245.

Anal. Calcd for $C_{21}H_{27}NO_5 \cdot HCl$: C, 61.53; H, 6.88; N, 3.42. Found: C, 61.08; H, 6.92; N, 3.58.

Hasubanonine Methiodide.—Hasubanonine (65 mg) in anhydrous benzene (2 ml) was treated with 0.5 ml of CH_3I (Aldrich) and the reaction was maintained at reflux for 2 hr. The crystalline material which was separated was recrystallized, first from methanol–ether and then methanol–water, to give colorless prisms (55 mg): mp 171–173° (lit.[20] mp 178°); $[\alpha]^{27}D$ −61° (*c* 0.28, methanol); λ_{max}^{EtOH} 264 mμ (ε 11,900); λ_{max}^{KBr} 2.84, 2.89, 3.39, 5.95, 6.18, 6.71 μ.[21]

(19) M. Tomita, T. Ibuka, Y. Inubushi, Y. Watanabe, and M. Matsui, *Tetrahedron Lett.*, 2937 (1964).

(20) H. Kondo, M. Satomi, and T. Odera, *Ann. Rept. Itsuu Lab.*, **2**, 1 (1951); H. G. Boit, "Ergebnisse der Alkaloid-Chemie Bis 1960," Academie-Verlag, Berlin, 1961.

(21) NOTE ADDED IN PROOF.—Since submission of the manuscript, we have isolated and characterized 4-demethylnorhasubanonine, $C_{19}H_{23}NO_5$,[6b] mp 116–119° (chloroform–ether); $[\alpha]^{25}D$ −219° (*c* 1.30, methanol); λ_{max}^{MeOH} 266 mμ (ε 11,750); λ_{max}^{KBr} 3.02, 6.02 μ; M⁺, *m/e* 345. Oxalate: mp 192–193° (methanol–ether); $[\alpha]^{25}D$ −159° (*c* 1.34, methanol); λ_{max}^{MeOH} 232 mμ sh (ε 11,185), 264 (9320); λ_{max}^{KBr} 2.89, 5.86, 5.98 μ. N-Methylation with methyl iodide gave Ia, characterized by ir, nmr, and tlc comparison with 4-demethylhasubanonine. The melting point of the oxalate salt was not depressed by admixture of authentic Ia oxalate.

Registry No.—Ia oxalate, 17968-59-1; Ia $HClO_4$, 18026-68-1; Ib, 17968-58-0; Ic oxalate, 18006-26-3; Ic HCl, 18006-27-4; Ic MeI, 18006-28-5.

A Small-Scale Synthesis of Mevalonolactone and Its 3-Ethyl-2-^{14}C Homolog

WILLIAM F. GRAY, GARY L. DEETS, AND THEODORE COHEN

Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

Received July 15, 1968

Mevalonic acid (or its lactone, 3,5-dihydroxy-3-methylpentanoic acid γ-lactone, **2b**) has long been recognized as a precursor of the isoprene unit used by living systems in biosynthesis.[1] Analogs of mevalonolactone, however, have not been extensively tested in biological systems. Tamura, *et al.*,[2] and Stewart and Woolley[3] have synthesized several homologs and tested them for antimetabolic activity, but the question whether 3,5-dihydroxy-3-ethylpentanoic acid γ-lactone (**2a**, the 3-ethyl homolog of mevalonolactone) is metabolized by living systems was left unanswered. In order to obtain material for such a study,[4] we undertook the synthesis of **2a** labeled with ^{14}C in the 2 position.

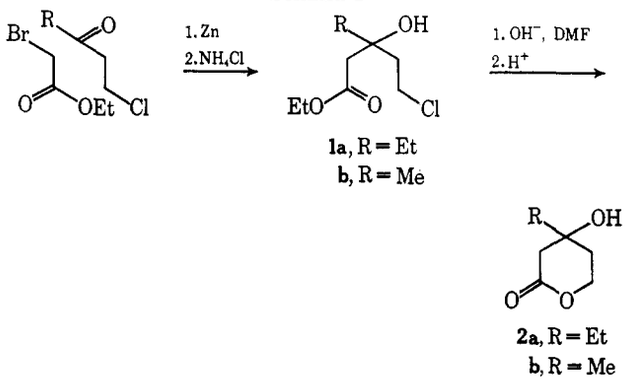
Compound **2a** has been synthesized by Tamura and Takai[5] by the method used for mevalonolactone.[6] The tetrahydropyranyl ether of the appropriate 3-keto alcohol was treated with allylmagnesium bromide; the protecting group was removed; the terminal olefin was cleaved by ozonolysis; and the lactone was obtained upon work-up, during which cyclization of the dihydroxy acid occurred.

Several other mevalonolactone syntheses have been reported. Those based on Hoffman's synthesis[7] involve a Reformatski reaction between ethyl bromoacetate and 4-acetoxy-2-butanone.[8-10] After saponification of the resulting diester, the lactone forms upon acidification. Hulcher and Hosick[11] reported an internal Reformatski reaction of 4-(bromoacetoxy)-2-butanone prepared from bromoacetyl bromide and 4-hydroxy-2-butanone. Cornforth and coworkers have reported syntheses of mevalonolactone labeled in both the 4 position and methyl group, and the 4 position alone.[12] They have also synthesized stereospecifically the (+) and (−) forms from (−)- and (+)-linalool, respectively.[13]

For the small-scale preparation of radioactively labeled **2a** or **2b**, each of the above sequences suffers from one or more of the following deficiencies: (a) three or more steps in the reaction scheme, (b) relatively low over-all conversion, (c) commerical unavailability of one of the starting materials. In addition, the final product is distilled in each of these procedures, thus making it desirable to devise another method of purification for small-scale work. We have therefore developed the procedure described below.

Compounds **2a** and **2b** have been prepared by the sequence shown in Scheme I. Where R = Et, the

SCHEME I



yield of purified product in each step is generally about 65–70%.[14] This procedure has the advantages that the sequence consists of only two steps, the starting materials are commercially available, the use of tlc in purification permits very small-scale reactions, and the product can be labeled at the 1 or 2 positions *via* the ethyl bromoacetate and at the 3 position or alkyl group *via* the acid chloride used in preparation of the chloro ketone.[15,16]

Experimental Section

Infrared spectra were determined with a Beckman Model IR-8 or a Perkin–Elmer Model 237-B spectrophotometer. The nmr spectra were determined with a Varian Model A-60 spectrometer. The chemical shifts are expressed in τ values relative to tetramethylsilane as an internal standard. Gross appearance of the peaks is reported, though some signals show higher order splitting. Mass spectra were obtained with an LKB-9000 combined gas chromatograph–mass spectrometer.[17] All spectra reported, with the exception of the ir spectrum of **2a**, are of nonradioactive materials. Preparative tlc was conducted using air-dried, unactivated[18] Mallinckrodt TLC-7GF[19] silicic acid. The 1-chloro-3-pentanone and 4-chloro-2-butanone were purchased from Aldrich and Chemical Procurement Labora-

(1) R. B. Clayton, *Quart. Rev.* (London), **19**, 168, 201 (1965).
(2) S. Tamura, G. Tamura, M. Takai, S. Nakamura, and T. Shiro, *Bull. Agr. Chem. Soc. Jap.*, **22**, 202 (1958).
(3) J. M. Stewart and D. W. Woolley, *J. Amer. Chem. Soc.*, **81**, 4951 (1959).
(4) This study is being carried out by Dr. C. C. Sweeley and his students.
(5) S. Tamura and M. Takai, *Bull. Agr. Chem. Soc. Jap.*, **21**, 394 (1957).
(6) S. Tamura and M. Takai, *ibid.*, **21**, 260 (1957).
(7) C. H. Hoffman, A. F. Wagner, A. N. Wilson, C. H. Shunk, D. E. Wolf, F. W. Holly, and K. Folkers, *J. Amer. Chem. Soc.*, **79**, 2316 (1957).
(8) O. Isler, R. Rüegg, J. Würsch, K. F. Gey, and A. Pletscher, *Helv. Chim. Acta*, **40**, 2369 (1957).
(9) J. Cornforth, R. Cornforth, C. Popjak, and I. Youhotsky-Gore, *Biochem. J.*, **69**, 146 (1958).
(10) A. L. Remizov and G. A. Tsvetkova, *Sintez Prirodn. Soedin., ikh Analogov i Fragmentov, Akad. Nauk SSSR, Otd. Obschi. i Tekhn. Khim.*, 129 (1965); *Chem. Abstr.*, **65**, 614a (1966).
(11) F. H. Hulcher and T. A. Hosick, U. S. Patent 3,119,842 (1964); *Chem. Abstr.*, **60**, 10554g (1964).
(12) J. W. Cornforth, R. H. Cornforth, A. Pelter, M. G. Horning, and G. Popjak, *Tetrahedron*, **5**, 311 (1959).
(13) R. H. Cornforth, J. W. Cornforth, and G. Popjak, *ibid.*, **18**, 1351 (1962).
(14) In the single preparation of mevalonolactone (R = Me), the hydrolysis step went in lower yield (52%).
(15) F. Sondheimer and R. B. Woodward, *J. Amer. Chem. Soc.*, **75**, 5438 (1953).
(16) F. F. Blicke and F. J. McCarty, *J. Org. Chem.*, **24**, 1376 (1959).
(17) We wish to thank the National Institutes of Health for the grant with which this instrument was purchased, and Dr. C. C. Sweeley and Mr. John Naworal for the spectra.
(18) The use of freshly prepared and activated layers can cause extensive dehydration during attempted purification of the lactone.
(19) In this system, cleaner products are obtained from Mallinckrodt TLC-7GF silicic acid than from Merck silica gel G.