tration showed primarily isomer **28b** and only a trace of **28a** by TLC (above). Chromatography on alumina (neutral, activity III) and elution with ethyl acetate–hexane (1:4) gave 151 mg (63%) of isomer **28b**. Equilibration of 15 mg of pure isomer **28b** under the same conditions showed a small amount of isomer **28a** by TLC.

16α- and 16β-(Carbomethoxy)-20-epivelbanamines (28c,d). Reduction of 490 mg (1.38 mmol) of *dl*-20-epipandoline with 400 mg (10.6 mmol) of sodium borohydride in 6 mL of acetic acid for 10 min, as above, gave 208 mg (42%) of the 16α-carbomethoxy isomer **28c** on crystallization from methanol and another 65 mg (13%) after chromatography of the mother liquors on alumina (neutral, activity III), eluting with 4:1 hexane–ethyl acetate, in addition to 33 mg (6.7%) of isomer **28d**. Two further minor products were detected by TLC, giving orange color reactions with CAS spray like the *N*-ethyl-2,16-dihydropandoline (**29a**).

Isomer **28c** (methanolate): recrystallized from methanol, mp 203–206 °C; TLC (Merck silica, dichloromethane–5% methanol), R_f 0.7, grey-green with CAS spray; UV (ethanol) λ_{max} (log ϵ) 230 (4.36), 286 (3.86), 291 (3.83) nm; IR (KBr) ν_{max} 3550, 3375, 1725, 1460, 1435, 1330, 1315, 1260, 1200, 1170, 1040, 1010, 930, 800, 750 cm^{-1}; NMR ($CDCl_3$) δ 8.62 (1 H, br s), 7.54–6.98 (4 H, m), 5.04 (1 H, d), 3.68 (3 H, s), 3.45 (CH_3OH), 0.93 (3 H, t); mass spectrum identical with that of **28a**. Anal. Calcd for $C_{21}H_{28}N_2O_3 \cdot CH_3OH$: C, 68.01; H, 8.30; N, 7.21. Found: C, 68.14; H, 8.24; N, 7.05.

Isomer **28d**: perchlorate, recrystallized from methanol, mp 238–241 °C; TLC (as above), R_f 0.3, green with CAS spray; UV (HCl salt, ethanol) λ_{max} (log ϵ) 228 (4.44), 285 (4.0), 2.93 (3.97); IR ($HClO_4$ salt, KBr) ν_{max} 3510, 3390, 3170, 1730, 1720 (shoulder), 1460, 1440, 1340, 1305, 1290, 1230, 1165, 1105, 1060, 1025, 935, 905, 840, 750 cm^{-1}; NMR ($CDCl_3$ free base) δ 8.92 (1 H, br s), 7.48–6.92 (4 H, m), 3.84 (1 H, dd), 3.64 (3 H, s), 0.71 (3 H, t); mass spectrum, as for **28c** except for decreased fragment peaks at *m/e* 257, 143, 124 and an increased peak at *m/e* 226 for **28d**.

Equilibration of the seco 20-epipandolines **28c,d** under the conditions described for the seco pandolines **28a,b** showed analogous conversions but a relatively decreased rate of conversion of **28c** to **28d**.

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Registry No. (±)-**5b**, 73837-57-7; (±)-**5c**, 73836-92-7; (±)-**5d**, 73824-79-0; (±)-**5e**, 73805-38-6; (±)-**8a**, 66859-30-1; (±)-**8b**, 66859-22-1; **8c**, 69069-71-2; **9b**, 69069-58-5; (±)-**10a**, 73816-12-3; (±)-**10b**, 73805-39-7; (±)-**10b** DNP, 73805-40-0; (±)-**18**, 66757-48-0; (±)-**19**, 73805-41-1; (±)-**20**, 73805-42-2; (±)-**21**, 73805-43-3; (±)-**22a**, 73805-44-4; (±)-**22b**, 73805-45-5; (±)-**23**, 73805-46-6; (±)-**24**, 73805-47-7; **25**, 73805-48-8; (±)-**27**, 73805-49-9; (±)-**28a**, 73836-93-8; (±)-**28b**, 56596-08-8; (±)-**28b** perchlorate, 73836-94-9; (±)-**28c**, 73836-95-0; (±)-**28d**, 73837-58-8; (±)-**28d** perchlorate, 73889-50-6; (±)-**29a**, 73816-13-4; diphenyl diselenide, 1666-13-3; 4-ethyl-4-pentenoic acid, 13722-73-1.

Secotropane Alkaloids of *Physalis peruviana*

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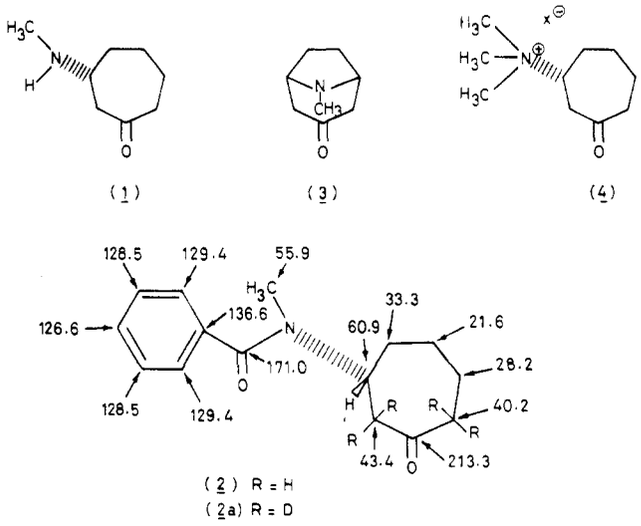
(+)-Physoperuvine, (±)-physoperuvine, and (+)-*N,N*-dimethylphysoperuvinium salt (anion unknown) were isolated from the roots of *Physalis peruviana*. Their structures were advanced on the basis of spectral and chemical evidence. These alkaloids comprise the first group of biogenetically interesting secotropane alkaloids.

The genus *Physalis* is reputed for elaborating C_{28} steroidal lactones, physalins and withanolides.[1] In addition to the isolation[2-5] of these groups of compounds from the leaves of *Physalis peruviana* L. (Solanaceae family), the presence of the alkaloids[6] tigloidine and 3-α-(tigloyloxy)-tropane was also detected in the roots of this plant. In the present paper we report that systematic fractionation of the alkaloidal constituents of the roots of *P. peruviana* yielded three alkaloids, none of which corresponded to either of the alkaloids[6] earlier reported from this source, and these were proved to be altogether new alkaloids. In a previous paper[7] we reported the structure of physoperuvine (1), the major alkaloid of this plant, exclusively on



^1H NMR and mass spectral evidence. We present here the detailed data in support of the structure and absolute configuration of physoperuvine and two of its congeners which comprise a new group of alkaloids and may appro-

(1) E. Glotter, I. Kirson, D. Lavie, and A. Abraham in "Bio-organic Chemistry", Vol. II, E. E. van Tamelen, Ed., Academic Press, New York, 1978, p 57.
(2) I. Kirson, A. Abraham, P. D. Sethi, S. S. Subramanian, and E. Glotter *Phytochemistry*, **15**, 340 (1976).
(3) K. Sakurai, H. Ishii, S. Kobayashi, and T. Iwas *Chem. Pharm. Bull.*, **24**, 1403 (1976).
(4) A. B. Ray, M. Sahai, and B. C. Das *J. Indian Chem. Soc.*, **55**, 1175 (1978).
(5) T. K. Bhattacharya, T. N. C. Vedantham, S. S. Subramanian, and I. Kirson *Indian J. Pharm. Sci.*, **40**, 177 (1978).
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(7) A. B. Ray, M. Sahai, and P. D. Sethi *Chem. Ind. (London)*, 454 (1976).

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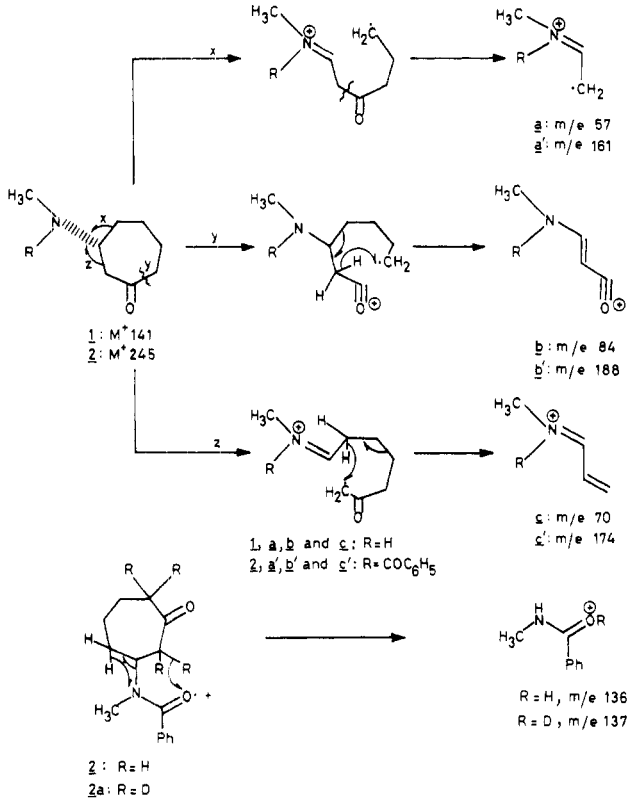
Scheme I. Mass Spectral Fragmentation Pathways of Physoperuvine (1), *N*-Benzoylphysoperuvine (2), and *N*-Benzoylphysoperuvine-d_4 (2a)



priately be termed as secotropanes.[8]

(+)-Physoperuvine (1) was crystallized from dry acetone as deliquescent needles: mp 152–153 °C; $[\alpha]_D$ +1.2°. Analytical results and mass spectrometric molecular weight determination (M^+, 141) established its molecular formula as $C_8H_{15}NO$. The alkaloid was recognized as a secondary amino ketone from its infrared spectral bands at 3300 (NH) and 1698 cm^{-1} (ketone) and from the observation that it furnished an *N*-benzoyl derivative (2) which in turn gave a crystalline oxime: $C_{15}H_{20}N_2O_2$ (M^+, 260); mp 98 °C. The ^1H NMR spectrum of *N*-benzoylphysoperuvine [$C_{15}H_{19}NO_2$ (M^+, 245), mp 136–137 °C, $[\alpha]_D$ +95.6°] showed signals associated with five aromatic protons (δ 7.36, 5 H, s), an *N*-methyl group (δ 2.86, 3 H, s), and a methine proton (δ 4.35, 1 H, br) in addition to two sets of methylene signals centered around δ 1.92 (6 H, br) and 2.46 (4 H, br). Brief reflux of 2 with alkaline D_2O gave a tetradeuterio derivative (**2a**), the ^1H NMR spectrum of which differed from that of the former only by the absence of four-proton methylene signals around δ 2.46 and thus indicated the presence of a CH_2COCH_2 system in physoperuvine. The structural units of the alkaloid were thus ascertained to be CH_2COCH_2, $CHNHCH_3$, and three CH_2's, and further, the alkaloid was proved to be monocyclic from valency calculation. Of the several structures that can be built up by assembling these units, β-methylaminocycloheptanone (1) was considered to be the most logical one not only on the basis of mass spectral peaks (Scheme I) but also in view of its structural resemblance with tropinone (3) or other tropane alkaloids which are quite common in the family Solanaceae. The correctness of this structure was also verified by ^{13}C NMR spectral analysis of 2. The chemical shifts (in parts per million) of different carbon resonance signals are shown in the

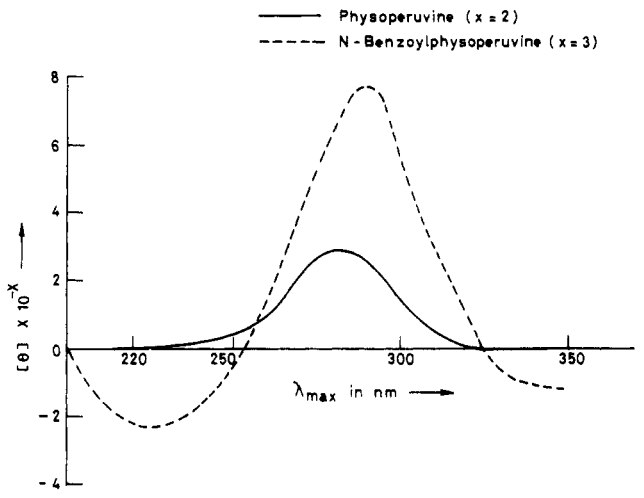


Figure 1. CD curves of physoperuvine (1) and *N*-benzoylphysoperuvine (2).

structure. Conclusive evidence in support of 1 came from the synthesis of *N,N*-dimethylphysoperuvinium iodide (4, X = I) by Michael-type addition of dimethylamine to 2-cyclohepten-1-one and subsequent quaternization of the resultant base with methyl iodide. The synthetic sample was indistinguishable from *N,N*-dimethylphysoperuvinium iodide, prepared by refluxing a solution of 1 in dry acetone with methyl iodide in presence of potassium carbonate. Both samples showed identical IR and ^1H NMR spectra and also the same melting point, indicating that the synthetic sample presumably exists as a racemic solid solution. Both physoperuvine (1) and its *N*-benzoyl derivative (2) showed positive Cotton effects around 290 nm (Figure 1) which were the same as that reported for (*R*)-(+)-3-methylcycloheptanone.[9] On the basis of this observation, the monosubstituted cycloheptanone derivative physoperuvine was proved to have the *R* configuration.

Following the (+)-physoperuvine from the chromatographic column was some (±)-physoperuvine which was isolated as a noncrystallizable, viscous oil. It showed the same molecular ion peak and mass fragmentation pattern as those observed for physoperuvine and gave a crystalline benzoyl derivative [$C_{15}H_{19}NO_2$ (M^+, 245), mp 137 °C] identical in all respects with 2, with the only difference being that its specific rotation was observed to be ±0°.

Precipitation of the total water-soluble base fraction as its Mayer's complex, treatment of the aqueous suspension of the complex with anion-exchange resin (IRA 400, Cl^- form), and subsequent chromatography of the concentrated aqueous extract led to the isolation of a crystalline quaternary base chloride [$C_{10}H_{20}NOCl$, mp 257–258 °C, $[\alpha]_D$ +88.8° (H_2O)] which showed a distinct keto carbonyl absorption at 1702 cm^{-1} and displayed a Zimmermann color reaction like physoperuvine. Hofmann elimination of quaternary base gave trimethylamine which was trapped as its ethiodide: $C_5H_{14}NI$; mp 316–318 °C. The ^1H NMR spectrum of the ethiodide showed a nine-proton singlet at δ 3.19 for three *N*-methyls, a three-proton triplet of triplets[10] (J = 7 and 2 Hz) at δ 1.43 for the methyl of the ethyl group adjacent to the quaternary nitrogen, and a two-proton quartet with fine splitting (J = 7 Hz) at δ 3.5 for the methylene group. The nonnitrogenous part of the Hofmann degradation product was obtained as an intractable gum, presumably due to extensive polymerization, and it could not be characterized. The molecular

(8) R. L. Clarke in "The Alkaloids", Vol. XVI, R. H. F. Manske, Ed., Academic Press, 1977, p 94.

(9) C. Djerassi, B. F. Burrow, C. G. Oberberger, T. Takakoshi, D. C. Gutsche, and T. C. Chang *J. Am. Chem. Soc.*, **85**, 949 (1963).

(10) P. G. Gassmann and D. C. Heckert *J. Org. Chem.*, **30**, 2859 (1965).

formula of the compound taken in conjunction with the presence of a trimethylammonium side chain and a carbonyl function in the molecule suggested that it was *N*,*N*-dimethylphysoperuvinium chloride (**4**, X = Cl). This supposition was verified by conversion of *N*,*N*-dimethylphysoperuvinium iodide to the corresponding chloride salt by treatment with ion-exchange resin (IRA 400, Cl^- form), and the two compounds were found to be indistinguishable from each other. Since the quaternary salt isolated was converted to a chloride salt in the process, the natural alkaloid is thus represented by **4** with the identity of X unknown.

The alkaloids, though akin to tropinone, are quite interesting from a biogenetic viewpoint. The present knowledge of the biogenesis[11] of tropane bases indicates that these alkaloids cannot be intermediates and are probably formed by fission of the bicyclic tropinone through Hofmann-type elimination and subsequent reduction of the double bond.

Experimental Section

Melting points are uncorrected. ^1H NMR spectra were determined in the stated solvents by using a Varian A-60D instrument unless otherwise stated; the chemical shifts are reported in parts per million and coupling constants (J) in hertz. IR spectra were recorded on a Perkin-Elmer spectrometer, Model 257, as Nujol mulls and UV spectra were recorded in spectral methanol on a Cary 14 spectrophotometer. Optical rotations were measured with JASCO Dip 180 photoelectric polarimeter and CD spectra on a Durrum-JASCO ORD/UV-5 spectropholarimeter with a Sproul Scientific SS-20 modification. TLC was performed on silica gel G (Centron) and column chromatography on silica gel (BDH) with stated solvents. Roots of *Physalis peruviana* were collected from the fields in the suburbs of Varanasi, India, and a voucher specimen is on file.

Extraction of *P. peruviana* Roots and Initial Fractionation. Ground roots (2.5 kg) were percolated to exhaustion with ethanol (95%), and the extract was evaporated to dryness at reduced pressure. The residue was suspended in 5% aqueous citric acid, stirred mechanically for 3 h, and then filtered. The filtrate was washed with an equal volume of $CHCl_3$ and then brought to pH 9.0 with NH_4OH. The basic solution was extracted exhaustively with $CHCl_3$ (1.5 L) in a liquid–liquid extractor. The $CHCl_3$ extract was concentrated to a small volume (10 mL) as a viscous oil (fraction A). The aqueous ammonia left after $CHCl_3$ extraction was acidified with dilute HCl, and the water-soluble alkaloids present in this fraction were precipitated as Mayer's complex by addition of Mayer's reagent. The light gray precipitate was filtered, washed free from excess of reagent, suspended in ion-free water, and stirred with IRA 400 (Cl^-) resin until the exchange was complete. The clear aqueous solution was separated from the resin and concentrated to a brown gum (fraction B) in a rotary vacuum evaporator.

Chromatography of Fraction A. Fraction A was chromatographed on silica gel (200 g), collecting fractions of 0.1 L for analysis. The column was initially eluted with EtOAc (1 L), followed by a mixture of MeOH in EtOAc (10%).

Physoperuvine (1). The residue (2.9 g) from fractions 16–23 was crystallized from dry acetone to yield 2.5 g of colorless crystalline physoperuvine (**1**): mp 153 °C; R_f 0.58 on TLC with *n*-BuOH–MeOH–NH_4OH–H_2O (2:2:1:1); $[\alpha]_D$ +1.2° (*c* 1.30, H_2O); $[\theta]_{284}$ +293; UV λ_{max} 273 nm (ϵ 12.3); IR 3300 (NH), 1698 cm^{-1} (ketone); ^1H NMR (D_2O) 2.1 (br, 5 CH_2), 2.76 (s, 3 H, NMe), 3.86 (br, 1 H, CHNHR); mass spectrum, *m*/*e* (relative intensity) 141 (36, M^+), 113 (24), 98 (39), 84 (39), 70 (100), 57 (85).

***N*-Benzoylphysoperuvine (2).** A mixture of physoperuvine (0.2 g), Et_3N (1 mL), and PhCOCl (1 mL) was kept overnight, and the reaction mixture was worked up in the usual manner to afford *N*-benzoylphysoperuvine (0.21 g) which crystallized from benzene as colorless needles: mp 136 °C; $[\alpha]_D$ +95.6° (*c* 1.3, $CHCl_3$); CD $[\theta]_{288}$ +7809; IR 1700, 1628 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.92 (br, 6 H, 3 CH_2), 2.46 (br, 4 H, 2 CH_2), 2,86 (s, 3 H, NMe), 4.36 (br, W_H = 15 Hz, 1 H, CHNCOPh), 7.36 (s, 5 H, Ar H); mass spectrum, *m*/*e* (relative intensity) 245 (44, M^+), 217 (9), 202 (3), 188 (10), 174 (24), 161 (22), 140 (19), 136 (99), 105 (100), 77 (97).

Anal. Calcd for $C_{15}H_{19}NO_2$: C, 73.46; H, 7.75; N, 5.71. Found: C, 73.50; H, 8.10; N, 5.43.

***N*-Benzoylphysoperuvine-d_4 (2a).** *N*-Benzoylphysoperuvine (0.2 g), which is soluble in hot water, was warmed with 0.1 N KOH in D_2O on a water bath for 2 h. The reaction mixture was cooled and extracted with ether. The ether extract was washed, dried, and evaporated to give a white residue (0.19 g) which was crystallized from benzene as colorless needles of **2a**: mp 136 °C; ^1H NMR ($CDCl_3$) δ 1.92 (br, 6 H, 3 CH_2), 2.86 (s, 3 H, NMe), 4.36 (br, 1 H, CHNCOPh), 7.36 (s, 5 H, Ar H); mass spectrum, *m*/*e* (relative intensity) 249 (40, M^+), 220 (5), 206 (2), 190 (8), 176 (13), 163 (3), 161 (12), 160 (15), 144 (12), 137 (50), 105 (100), 77 (97).

Preparation of the Oxime of *N*-Benzoylphysoperuvine. To a mixture of *N*-benzoylphysoperuvine (0.27 g), hydroxylamine hydrochloride (0.12 g), ethanol (4 mL), and water (1 mL) was added powdered NaOH (0.22 g) in portions at room temperature. The mixture was refluxed for 10 min and then poured into 4 N HCl (10 mL). The solution was freed from EtOH and then partitioned between H_2O and $CHCl_3$. The $CHCl_3$ extract was washed, dried, and evaporated to give a solid residue (0.22 g) which crystallized from Me_2CO as colorless needles: $C_{15}H_{20}N_2O_2$; mp 98 °C; IR (KBr) 1628 (benzamide), 1610 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.72 (br, 6 H, 3 CH_2), 2.45 (br, 4 H, 2 CH_2), 2.76 and 2.98 (2 s, 1.5 H each, NMe for two geometrical isomers), 3.30 and 4.40 (br, 0.5 H each, CHN), 7.33 (s, 5 H, Ar H), 8.55 (s, 1 H, =NOH); mass spectrum, *m*/*e* (relative intensity) 260 (7, M^+), 243 (74), 212 (36), 184 (9), 174 (8), 155 (32), 136 (36), 122 (14), 111 (33), 105 (100), 77 (97).

Preparation of (+)-*N*,*N*-Dimethylphysoperuvinium Iodide from (+)-Physoperuvine. To a solution of (+)-physoperuvine (0.5 g) in dry Me_2CO (150 mL) were added anhydrous K_2CO_3 (5 g) and CH_3I (4 mL), and the reaction mixture was refluxed for 1.5 h and filtered hot to remove the inorganics. The filtrate on concentration gave colorless needles of **4** (X = I): mp 288 °C; IR (KBr) 1702 cm^{-1}; ^1H NMR (D_2O) δ 2.1 (br, 10 H, 5 CH_2), 3.15 (s, 9 H, NMe_3), 3.83 (br, 1 H, CHN).

Anal. Calcd for $C_{10}H_{20}NOI$: C, 40.40; H, 6.74; N, 4.71. Found: C, 40.91; H, 6.87; N, 4.35.

Total Synthesis of (±)-*N*,*N*-Dimethylphysoperuvinium Iodide (4, X = I). To a 2 M aqueous solution of dimethylamine was added 2-cyclohepten-1-one (0.8 g) in MeOH (5 mL), and the mixture was kept at room temperature for 1 h. MeOH was evaporated off, and the residue was partitioned between $CHCl_3$ and 5% aqueous AcOH. The acid solution was basified (NH_4OH) and extracted with $CHCl_3$, and the $CHCl_3$ extract was washed, dried (Na_2SO_4), and evaporated to give a basic gum (0.44 g) which was chromatographed over Al_2O_3. Elution with CH_2Cl_2 afforded a homogeneous oil which was taken in Me_2CO, treated with excess of CH_3I (2 mL), and kept in a refrigerator overnight. The separated crystals were filtered and crystallized from acetone as needles (mp 287–288 °C), indistinguishable from the corresponding product described above from physoperuvine (co-TLC, IR, ^1H NMR, melting point).

(±)-Physoperuvine. The residue (0.25 g) from column fractions 25–28 was obtained as a reddish brown gum which was filtered through an alumina column to obtain a light yellow homogeneous liquid: R_f 0.58 on TLC plates with *n*-BuOH–MeOH–NH_4OH–H_2O (2:2:1:1); $[\alpha]_D$ ±0° (*c* 1.3, H_2O). Benzoylation of this homogeneous liquid by the usual procedure yielded a crystalline solid [mp 136–137 °C, $[\alpha]_D$ ±0° (*c* 1.3, $CHCl_3$)] which was indistinguishable from **2** in spectral properties (IR, ^1H NMR, and mass spectra) and TLC behavior.

***N*,*N*-Dimethylphysoperuvinium Chloride (4, X = Cl).** The crude quaternary alkaloid fraction (fraction B) was separated on a column of silica gel (200 g), collecting 0.1-L fractions for analysis. The eluting solvents were EtOAc (0.5 L), and the following mixtures of MeOH in EtOAc: 10% (0.7 L), 25% (0.9 L), 40% (1 L), and 75% (1 L). The residue from fractions 23–31 was obtained as a colorless solid which was crystallized from MeOH to yield 0.3 g of colorless needles of **4** (X = Cl): mp 255–258 °C; $[\alpha]_D$ +88.8° (*c* 1.3, H_2O); R_f 0.41 on TLC with *n*-BuOH–MeOH–

(11) G. Fodor in "The Alkaloids", Vol. XIII, R. H. F. Manske, Ed., Academic Press, 1971, p 384.

NH_4OH–H_2O (2:2:1:1); IR 1702 cm^{-1}; ^1H NMR (D_2O) δ 2.10 (br, 10 H, 5 CH_2), 3.15 (s, 9 H, NMe_3), 3.83 (br, 1 H, CHN).

Anal. Calcd for $C_{10}H_{20}NOCl$: C, 58.39; H, 9.75; N, 6.81. Found: C, 58.31; H, 9.92; N, 6.58.

Hofmann Degradation of 4 (X = Cl). A 0.2-g sample of **4** (X = Cl) was dissolved in 30 mL of aqueous ethanolic KOH (1 N), the solution was refluxed on a water bath for 2 h in an N_2 atmosphere, and the outgoing gas was bubbled through an ethanolic solution of EtI. Removal of solvent from the bubbler furnished a white solid (0.08 g) which crystallized from EtOH as needles: mp 316–318 °C; ^1H NMR (D_2O) 3.19 (s, 9 H, NMe_3), 1.43 (tt, J = 7 and 2 Hz, 3 H, $N^+CH_2CH_3$), 3.50 (q, J = 7 Hz, 2 H, $N^+CH_2CH_3$). The nonbasic fraction left in ethanolic alkali was obtained as a gum which could not be purified for identification.

Conversion of *N,N*-Dimethylphysoperuvinium Iodide to Quaternary Alkaloid 4 (X = Cl). *N,N*-Dimethylphysoperuvinium iodide (0.3 g) was dissolved in ion-free water (30 mL), and the solution was stirred with anion-exchange resin (IRA 400, Cl^- form) for 4 h. The aqueous solution was filtered from the resin and evaporated to dryness under reduced pressure. The solid residue was crystallized from MeOH as colorless needles: 0.12 g; mp 255–258 °C; $[\alpha]_D$ +86.2° (*c* 1.0, H_2O); indistinguishable from the chloride salt of the natural quaternary base (melting point, mixture melting point, co-TLC, IR).

Acknowledgment. We are grateful to Professor H. Hikino of Tohoku University, Japan, for the ^{13}C NMR spectral analyses and to Dr. B. C. Das of the Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France, for the mass spectral analyses. A gift sample of 2-cycloheptenone from Dr. M. T. Shipchandler is gratefully acknowledged. Thanks are due to Dr. J. V. Juvarkar, College of Pharmacy, Ohio State University, for CD spectra and to Professor B. Dasgupta of our department for his kind interest in the problem. M.S. is grateful to CCRAS and CSIR, New Delhi, for financial assistance.

Registry No. (+)-**1**, 60723-27-5; (±)-**1**, 73744-99-7; (+)-**2**, 60723-28-6; (±)-**2**, 73745-00-3; (+)-**2a**, 60723-29-7; (+)-**2** oxime isomer 1, 73697-49-1; (+)-**2** oxime isomer 2, 73697-52-6; (+)-**4** (X = I), 73697-50-4; (+)-**4** (X = Cl), 73697-51-5; (±)-**4** (X = I), 73745-01-4; dimethylamine, 124-40-3; 2-cyclohepten-1-one, 1121-66-0; (+)-**2** oxime isomer 2, 73697-52-6.

Structure of Elasnin, a Novel Elastase Inhibitor Containing an α-Pyrone Ring

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Elasnin ($C_{24}H_{40}O_4$), isolated from the cultured broth of *Streptomyces noboritoensis* KM-2753, is a new specific inhibitor of human granulocyte elastase. Evidences based on spectral analyses, chemical degradations, and biosynthetic means using a ^{13}C-labeled precursor established its structure as **1**, having a highly alkylated 4-hydroxy-α-pyrone, rather than a 2-hydroxy-γ-pyrone, as the most likely skeletal structure. Structural comparison between elasnin and its methyl ether obtained by CH_2N_2 treatment was investigated, using UV data and ^{13}C–^{13}C couplings in both compounds biosynthetically enriched with [1,2-^{13}C]acetate. Structural change from α-pyrone to γ-pyrone occured in the methylation of elasnin with CH_2N_2 but not in the acetylation with acetic anhydride in pyridine.

In our screening program for inhibitors of human granulocyte elastase, a new inhibitor designated as elasnin was isolated from the culture filtrate of *Streptomyces noboritoensis* KM-2753.[1,2] Elasnin markedly inhibits human granulocyte elastase, but it is almost inactive against pancreatic elastase, chymotrypsin, and trypsin.[3,4] The present paper deals with the structural elucidation of elasnin on the basis of chemical degradations and a biosynthetic investigation using a ^{13}C-labeled precursor and ^{13}C NMR spectroscopy.[5]

Elasnin (**1**) (Chart I) is a lipophilic colorless and viscous oil: $C_{24}H_{40}O_4$, mass spectrum, m/z (M^+, 392); $n^{17}{}_D$ 1.4983; $[\alpha]^{18}{}_D$ –0.9° (*c* 1, EtOH); UV (EtOH) λ_{max} 291 nm (ϵ 7760). The IR (CCl_4) spectrum of **1** exhibited characteristic absorption bands due to methylenic and methyl groups at 2960 and 2860 cm^{-1}, a ketone carbonyl at 1715 cm^{-1}, a conjugated ester carbonyl at 1665 cm^{-1}, and a double bond at 1636 cm^{-1}. The 25.2-MHz ^{13}C NMR spectrum of **1** shows the presence of a ketone carbonyl at δ 207.0, an ester carbonyl at either δ 165.5 or 164.7, and four quaternary olefinic carbons at either δ 165.5 or 164.7, 153.8, 115.0, and 104.3, as shown in Figure 4 (A). The appearance of four methyl carbons completely overlapped at δ 13.9, thirteen methylenic carbons at δ 22.4–40.3, and a methine at δ 54.7 suggests the existence[6] of four linear C_4 and C_5 alkyl chains in **1**.

Acetylation of **1** with acetic anhydride and pyridine afforded monoacetyl elasnin (**2**): $C_{26}H_{42}O_5$, mass spectrum, m/z (M^+, 434); UV λ_{max} 306.5 nm (ϵ 7200). The IR spectrum of **2** exhibited the absorption band for enolic ester carbonyl at 1775 cm^{-1}, indicating the presence of an enolic hydroxyl group in **1**. This was also confirmed from the formation of the methyl ether **3**: $C_{25}H_{42}O_4$, mass spectrum, m/z (M^+, 406); UV λ_{max} 252 nm (ϵ 7890); ^1H NMR δ 3.8 (s, 3 H OCH_3). **3** is obtained by the treatment

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