[8-Arginine]-vasopressin, L-Half-cystinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-L-half-cystinyl-L-prolyl-L-arginylglycinamide 1,6-Disulfide, III. Recrystallized protected nonapeptide I (73.2 mg, 46 μmol) was dissolved in liquid ammonia (150 ml) which had been distilled from sodium. Sodium was added in small quantities to the boiling ammonia solution over a period of 15–20 min until a permanent blue color remained for 30 sec, approximately 10 mg of sodium being necessary. Glacial acetic acid (2 drops) was added, the ammonia was evaporated to a small volume, and the remainder removed from the frozen state (at approximately −77°) at water pump vacuum. The lyophilisate was dissolved in deoxygenated 0.2 *N* acetic acid (200 ml) under nitrogen. The pH was adjusted to 6.8 with 0.5 *N* ammonia and air was passed through the solution for 2 hr after which the pH was adjusted to 4.8 with acetic acid. A total of 18,000 units of rat pressor activity was obtained. The solution was applied to an IRC-50 column (2 × 7 cm) in the H^+ form for desalting.[30] The column was washed with 0.25% acetic acid (250 ml) until the pH of the effluent reached approximately 3.5. The hormone was eluted with a pyridine–acetate solution (30 ml of pyridine and 4 ml of acetic acid diluted with water to 100 ml). The eluate was lyophilized to give a colorless powder (56.5 mg, 98% based on I) with a rat pressor activity of 270 units/mg. Following another deprotection of I (40 mg) a 90% yield of III (28.5 mg) with an activity of 350 rat pressor units/mg was obtained.

Purification of [8-Arginine]-vasopressin by Ion Exchange Chromatography. A preparation of III with 270 rat pressor units/mg (59 mg) was dissolved in 0.5 *M* ammonium acetate buffer at pH 6.4 (2 ml) and applied to a column (1.1 × 55 cm) of IRC-50 which had been equilibrated with the buffer at 4°. The column was eluted with the same buffer at 4° with a flow rate of 5 ml/hr; the main peak was preceeded by two small peaks. Lyophilization of the center portion of the main peak gave [8-arginine]-vasopressin (19 mg, 32%) possessing approximately 500 rat pressor units/mg. Lyophilized material from both sides of the main peak (18 mg, 31%) exhibited lower rat pressor activity; the total recovery of activity was 80%.

Purified III exhibited $[\alpha]^{22}D$ −22° (*c* 0.22, 1 *N* acetic acid); high voltage paper electrophoresis (3450 V, 65 V cm^{-1}, 40 min, room temperature) gave single spots: mobility, 11.5 cm at pH 2.1 ($HCOOH-CH_3COOH-H_2O$, 25:87:888 v/v), and 8.6 cm at pH 6.5 (pyridine–$CH_3COOH-H_2O$, 100:4:896 v/v); electrophoresis on cellulose polyacetate (200 V, 14.3 V cm^{-1}, 20 min, room temperature, 0.02 *M* sodium phosphate buffer pH 6.5) gave a single spot, mobility 0.8 cm. A sample for analysis was dried at 105° for 16 hr *in vacuo* (P_2O_5 and KOH).

Anal. Calcd for $C_{46}H_{65}O_{12}N_{15}S_2-2CH_3COOH-2H_2O$ (1240.4): C, 48.4; H, 6.26; N, 16.9; H_2O, 2.90. Found: C, 48.3; H, 6.16; N, 16.5; H_2O, 2.80 (Karl Fischer).

Amino acid analysis (6 *N* HCl, 105°, 24 hr) gave the following molar ratios, glycine being taken as 1.0: Arg, 1.02; Asp, 1.02; Glu, 1.08; Pro, 1.01; Gly, 1.0; Cys, 1.9; Tyr, 0.98; Phe, 1.03; NH_3, 2.9.

Bioassay Method. Assays for antidiuretic activity were performed on anesthetized, hydrated Sprague-Dawley male rats according to the method of Jeffers, Livezey, and Austin[40] as modified by Sawyer;[41] maximal depression of urine flow, in contrast to average duration of the response, was used to measure the antidiuretic activity. Assays were carried out on 12 rats; not more than six hormone injections[42] were given to each animal. Rat pressor assays were carried out on nine atropinized, urethane-anesthetized male rats as described in the United States Pharmacopeia.[43] Oxytocic assays were performed on six isolated uterine horns from three rats in natural estrus according to the method of Holton,[44] modified by Munsick[45] with the use of magnesium-free van Dyke–Hastings solution as the bathing fluid. The milk-ejecting activity was determined in three anesthetized, lactating rabbits following the procedure of Chan.[46] Avian vasodepressor assays were performed on four conscious chickens according to the procedure employed by Munsick, Sawyer, and van Dyke.[36] The biological activities were measured against the USP posterior pituitary reference standard; in all of these bioassays the four-point design was used and standard errors were calculated according to the method of Bliss.[47]

Acknowledgments. The authors are indebted to Dr. S. Farber (Boston) for his support, to Dr. H. Maeda (Boston) for the amino acid analyses, and to Mrs. I. Mintz (New York) and Miss M. Wahrenburg (New York) for bioassays.

(40) W. A. Jeffers, M. M. Livezey, and J. H. Austin, *Proc. Soc. Exp. Biol. Med.*, 50, 184 (1942).
(41) W. H. Sawyer, *Endocrinology*, **63**, 694 (1958).
(42) P. S. Tata and O. H. Gauer, *Pfluegers Arch.*, **290**, 279 (1966).
(43) "The Pharmacopeia of the United States," 17th Revision, Mack, Easton, Pa., 1965, p 750.
(44) P. Holton, *Brit. J. Pharmacol.*, **3**, 328 (1948).
(45) R. A. Munsick, *Endocrinology*, **66**, 451 (1960).
(46) W. Y. Chan, *J. Pharmacol. Exp. Ther.*, **147**, 48 (1965).
(47) C. I. Bliss, "The Statistics of Bioassay," Academic Press, New York, N. Y., 1952.

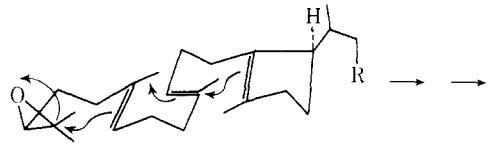
Communications to the Editor

Biogenetic-Type Synthesis of the Isoeuphenol System

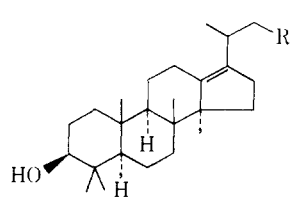
Sir:

As a further development in our continuing bioorganic studies of terpenoid terminal epoxides,[1] we wish to report the total synthesis of the isoeuphenol system (**3**), featuring the stereoselective generation of five asymmetric centers during cyclization of epoxide **1** (Mechanism A).[2] This step represents the closest nonenzymic approach thus far to a basic biosynthetic (all-chair) cyclization scheme[3]—modified (as in Mechanism B) to include the established intermediate,

Mechanism A



1, R = H
2, R = $CH_2CH{=}C(CH_3)_2$



3, R = H
4, R = $CH_2CH{=}C(CH_3)_2$

(1) E. E. van Tamelen, *Accounts Chem. Res.*, **1**, 111 (1968).
(2) For preceding examples of stereoselective, biogenetic-type polycyclizations of terpene terminal epoxides, see *e.g.*, E. E. van Tamelen, A. Storni, E. J. Hessler, and M. Schwartz, *J. Amer. Chem. Soc.*, **85**, 3295 (1963); E. E. van Tamelen and R. G. Nadeau, *ibid.*, **89**, 176 (1967).
(3) (a) G. Stork and A. W. Burgstahler, *ibid.*, 77, 5068 (1955); (b) A. Eschenmoser, L. Ruzicka, O. Jeger, and D. Arigoni, *Helv. Chim. Acta*, **38**, 1890 (1955).

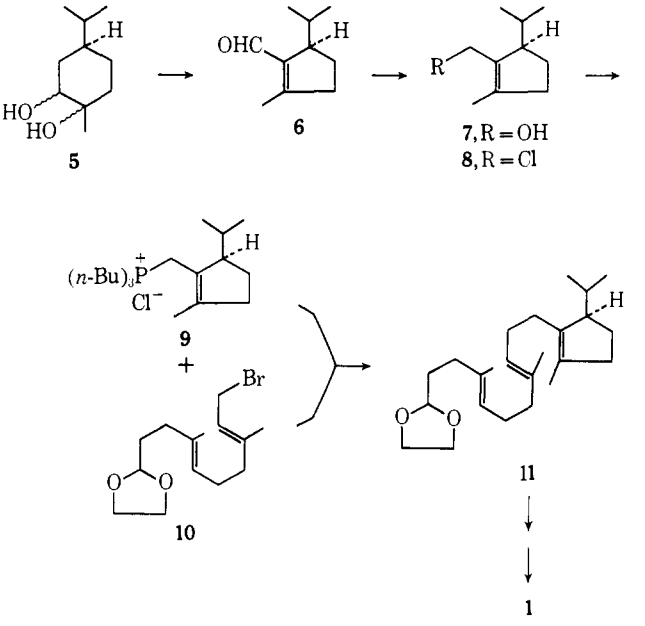
Mechanism B

squalene 2,3-oxide[4]—proposed to account for a vast array of polycyclic, squalene-derived triterpenes.

In order simultaneously to elaborate the desired tetracyclic framework (**3**) and achieve C-3 functionalization, a polyene terminal epoxide having a *preformed* D ring (**1**) was selected as the key intermediate. Because it is tetrasubstituted, the π bond in the D ring would permit control of carbonium ion behavior and consequent generation of a six-, rather than five-membered C ring.[5] Also, the construction of a tetracycle would be simplified, since only three new rings need then be formed in the cyclization process (Mechanism A). Finally, in the C_{30} series, where R=CH_2-$CH{=}C(CH_3)_2$, the established D ring should act as an "insulator" and deter involvement of the side-chain π bond with any carbonium ion developing during cyclization, a possibility inherent in a cyclization of the **B** type.

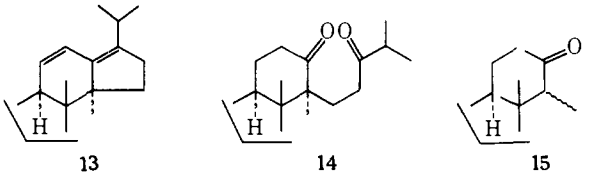
The synthesis of the monocyclic epoxide **1** was carried out by the coupling of a C_{10} unit (**8**) derived from *S*-(−)-limonene with farnesyl bromide trisnoracetal (**10**). Oxidation of 8,9-dihydro-*S*-(−)-limonene with peracetic acid gave the expected epoxide (ir 840 cm^{-1}) (95% yield), which on hydrolysis with 3% perchloric acid was converted to crystalline diastereomeric diol **5** (52%, mp 82–84°). The ketoaldehyde [ir 1720 cm^{-1}, broad; nmr δ 9.8 (1, t, J = 2 Hz)] prepared by the $NaIO_4$ cleavage of diol **5** was condensed in the presence of piperidine with water removal (Dean–Stark) to give α,β-unsaturated aldehyde **6** [76%; ir 1680 cm^{-1}; nmr δ 10.7 (1, s), 2.0 (3, s), 0.88 (3, d, J = 7 Hz), 0.65 (3, d, J = 7 Hz)], which was reduced to the corresponding allylic alcohol **7** (83%) with sodium borohydride. The allylic chloride **8** [nmr δ 2.6 (2, s), 1.6 (3, s), 0.92 (3, d, J = 7 Hz), 0.86 (3, d, J = 7 Hz)] was converted to the phosphonium chloride **9**, the ylide from which was coupled[6] with trisnoracetal **10**[7] to give, on reduction with lithium–ethylamine, monocyclic acetal **11** (55%).[8] The aldehyde [nmr (CCl_4) δ 9.7 (1, t, J = 2 Hz)], obtained by perchloric acid hydrolysis of **11**, was converted to desired epoxide **1**[9] by treatment with diphenylsulfonium isopropylide[10] (75%).

Treatment of epoxide **1** with BF_3–Et_2O or $SnCl_4$ in different solvents was found to give varying amounts of



tetracycle **3**. In the best run, 5 equiv of $SnCl_4$ in CH_3NO_2 provided a 70% yield of alcoholic material comprising at least seven products. The major optically inactive product, **3**, mp 126–128° (35%, based on **1**; *m/e* M^+ 438, $C_{25}H_{40}O$) was isolated in 95% purity by silica gel chromatography and further purified by crystallization and preparative glpc. The nmr spectrum contained no vinyl H or CH_3 resonances, indicating a tetracyclic structure for **3** with a tetrasubstituted double bond bearing no methyl substituent. The presence of ions at *m/e* 189, 190, and 191 in the mass spectrum, ascribed to ring AB fragments, rules out the presence of a Δ^8 double bond, as in euphol, but is in agreement with the assignment of a $\Delta^{13,17}$ bond, as in isoeuphol (**4**).

Treatment of sterol **3** with acetic anhydride in pyridine gave the corresponding sterol acetate, **12**, mp 132–134°. The recovery of this acetate after treatment with 30% HCl in acetic acid (conditions sufficient for the conversion of $\Delta^{13,17}$-protolanosterol acetate to lanosterol acetate)[11] was in agreement with the assignment of isoeuphenol structure and stereochemistry.[12] Oxidation of acetate **12** with peracid followed by acid-catalyzed dehydration gave diene **13** [uv (EtOH) 246, 254, 264 mμ (ϵ 19,500, 21,400, 14,100)], having a uv chromophore virtually identical with that observed for the 11,13-diene from isoeuphenol acetate [uv (EtOH) 246, 254, 264 mμ (ϵ 17,800, 22,200, 14,100)].[13] Hydrogenation over PtO_2 reconverted **13** to starting sterol acetate **12**.



(4) (a) E. E. van Tamelen, J. D. Willett, R. B. Clayton, and K. E. Lord, *J. Amer. Chem. Soc.*, **88**, 4752 (1966); (b) E. J. Corey and W. E. Russey, *ibid.*, **88**, 4750 (1966).

(5) E. E. van Tamelen, J. Willett, M. Schwartz, and R. Nadeau, *ibid.*, **88**, 5937 (1966).

(6) E. H. Axelrod, G. M. Milne, and E. E. van Tamelen, *ibid.*, **92**, 2139 (1970).

(7) K. B. Sharpless, R. P. Hanzlik, and E. E. van Tamelen, *ibid.*, **90**, 209 (1968).

(8) Nmr (CCl_4) δ 5.0–5.3 (3, broad), 4.75 (1, t, J = 6 Hz), 3.78–3.95 (4, m), 2.4–2.6 (1, broad), 1.8–2.4 (14, broad m), 1.59 (9, broad s), 0.92 (3, d, J = 7 Hz), 0.66 (3, d, J = 7 Hz).

(9) Nmr ($CDCl_3$) δ 5.15 (2, broad m), 2.66 (1, t, J = 6 Hz), 1.9–2.4 (14, broad m), 1.62 (9, broad s), 1.30 (3, s), 1.26 (3, s), 0.92 (3, d, J = 7 Hz), 0.66 (3, d, J = 7 Hz).

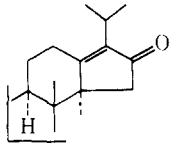
(10) E. J. Corey, M. Jautelat, and W. Oppolzer, *Tetrahedron Lett.*, 2325 (1967).

(11) Private communication from Professor D. Arigoni.

(12) The Δ^8 double bond of euphenol is unstable with respect to $\Delta^{13(17)}$-isoeuphenol in acidic media: K. Christen, M. Dünnenberger, C. B. Roth, H. Heusser, and O. Jeger, *Helv. Chim. Acta*, **35**, 1756 (1952).

(13) F. G. Fischer and N. Seiler, *Justus Liebigs Ann. Chem.*, **626**, 185 (1959).

Oxidation of acetate **12**, carried out with RuO_4 or with OsO_4 followed by $NaIO_4$,[12] gave 1,5-diketone **14**[14] in high yield. Heating with KOH in diethylene glycol gave the tricyclic retro-Michael product shown to be identical (except for optical activity) with the product derived from isoeuphol (**4**)[15] by ir, mass spectral, and vpc comparisons, thereby establishing the identity of the stereochemistry of **3** with that of isoeuphol (**4**) (with the exception of the epimerizable 14-methyl). Oxidation of **12** with dipyridyl chromium trioxide gave α,β-unsaturated ketone **16**, mp 132–133° [uv (EtOH) 243 mμ (ε 12,600); ir ($CHCl_3$) 1725, 1685, 1630 cm^{-1}] which on ozonolysis, oxidative work-up, treatment of the acidic products with diazomethane, and chromatography gave acetoxy keto ester **17**, mp 192–194°. The ir, nmr, and mass spectra of **17** were shown to be identical with those



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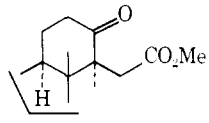


17

characteristic of the product obtained by degradation of isoeuphol.[15a] This set of results establishes without ambiguity the structure and stereochemistry of tetracycle **3** obtained by cyclization of monocyclic epoxide **1**.[16] The synthetic work herein, along with the earlier nonenzymic, selective terminal oxidation of squalene,[17] represents an overall, close simulation of the squalene → tetracyclic triterpene bioconversion and defines the purely organic chemical basis for operation of enzymes therein.

Acknowledgment. Financial support was provided by the National Science Foundation (GP 7187). The authors are also grateful to Professor D. Arigoni, Eidgenössische Technische Hochschule, for samples of euphol acetate and degradation products.

(14) Ir ($CHCl_3$) 1710 cm^{-1}; nmr ($CDCl_3$) δ 0.86 (3, s), 0.89 (6, s), 0.92 (3, s), 1.14 (3, s), 1.08 (3, d, J = 7 Hz), 1.095 (3, d, J = 7 Hz) (the isopropyl methyls are nonequivalent).

(15) (a) D. Arigoni, R. Viterbo, M. Dünnenberger, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, **37**, 2306 (1954). (b) For a discussion concerned with the revision of the originally assigned structure **15**, see G. V. D.-Modrone, Ph.D. Dissertation (No. 4156), Eidgenössische Technische Hochschule, Zurich, 1968.

(16) Satisfactory ir, nmr, uv, mass spectral, and analytical data were obtained for all synthetic intermediates.

(17) E. E. van Tamelen and T. J. Curphey, *Tetrahedron Lett.*, 121 (1962).

(18) NIH Postdoctoral Fellow, 1969–1970.

(19) NSF Predoctoral Fellow, 1967–1968.

* Address correspondence to this author.

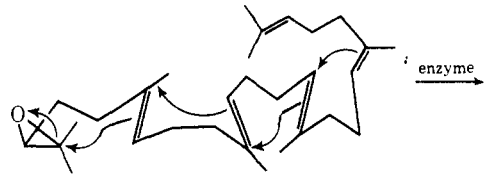
E. E. van Tamelen,* G. M. Milne,[18] M. I. Suffness[18]
M. C. Rudler Chauvin, R. J. Anderson,[19] R. S. Achini
Department of Chemistry, Stanford University
Stanford, California 94305
Received August 31, 1970

Formation of the Lanosterol System through Biogenetic-Type Cyclization

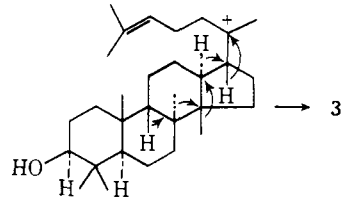
Sir:

Explicit in the Zurich proposal[1] for the biosynthesis of sterols is appearance of a "protolanosterol" intermediate (**2**) having, in addition to 8α and 14β methyls, the unusual 9β,10β (cis) relationship of hydrogen and methyl, a total arrangement logically permitting formation of lanosterol (**3**) structure and stereochemistry by means of a series of 1,2-methyl–hydrogen shifts and C-9 proton loss. This proposal, illustrated (Mechanism A) using the established natural substrate

Mechanism A



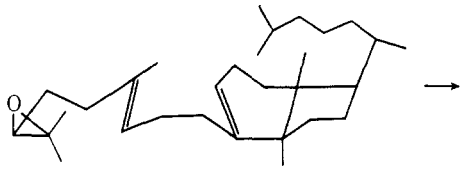
1



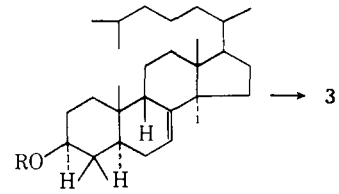
2

squalene 2,3-oxide (**1**),[2] requires the generation of a comparatively unstable ring B boat in intermediate **2**, generated by chair–boat–chair cyclization (Mechanism A). We wish to report that nonenzymic chair–boat cyclization (Mechanism B) of the polyene terminal

Mechanism B



4



5a, R = H
5b, R = CH_3CO

epoxide **4** produces a dihydrolanosterol isomer, shown to have the 9,10 cis structure **5**, which can be separately converted to Δ^8-dihydrolanosterol itself.

Epoxide **4** was obtained through use of a coupling reaction, the key component of which was prepared from lanosterol. Dehydrobromination (80%) of 2α-bromolanost-7-en-3-one[3] gave the Δ^1 ketone **6**: mp 123–125°; $[\alpha]^{20}D$ ($CHCl_3$) +28°; ir ($CHCl_3$) 1650 cm^{-1}; λ_{max}^{EtOH} 229 nm (ε 10,000); nmr (CCl_4) δ 5.30 (m, 7 H), 5.73 and 6.78 (dd, J = 10 Hz, 2 H and 1 H, respectively).

The desired cleavage of dienone **6** was effected by heating to 230–250° *in vacuo*, so that it distilled through a quartz column packed with glass helices and maintained at 600°.[4] The product was collected in a cooled

(1) A. Eschenmoser, L. Ruzicka, O. Jeger, and D. Arigoni, *Helv. Chim. Acta*, **38**, 1890 (1955).

(2) E. J. Corey and W. E. Russey, *J. Amer. Chem. Soc.*, **88**, 4750 (1966); E. E. van Tamelen, J. D. Willett, R. B. Clayton, and K. E. Lord, *ibid.*, **88**, 4752 (1966).

(3) Carried out according to the method used in the lanost-8-en-3-one series: D. H. R. Barton, D. A. Lewis, and J. F. McGhie, *J. Chem. Soc.*, 2907 (1957).