Anal. Calcd for $C_{19}H_{30}O$: C, 83.15; H, 11.01. Found: C, 83.12; H, 11.03.

Preparation of XVI.—Hydrogenation of XV (17 mg) in 2.5 ml of ethyl acetate in the presence of 5 mg of 5% Pt–C at room temperature and atmospheric pressure gave a quantitative yield of XVI, mp 146–148°, identical in all respects with the same compound prepared from III (R = CH_2OH) as previously described.[3]

Preparation of XVII.—A solution of 2.49 g of XV in 48 ml of anhydrous pyridine was added to a mixture of 3.8 g of chromic anhydride in 42 ml of anhydrous pyridine and the mixture was stirred at room temperature for 13 hr. The brown precipitate was removed by filtration and washed with pyridine. The pyridine wash was combined with the filtrate and 250 ml of ice water was added and the entire mixture was extracted with ether. The ether extract was washed with cold 5% HCl, then water, and finally dried over $MgSO_4$. Evaporation gave 2.37 g of semicrystalline product (λ_{max} 3.0–3.5 μ broad). An ether solution (50 ml) of this product was extracted with 10% NaOH and the alkaline layer, after extraction with ether, was acidified with 5% cold HCl. The ether extract of the acidified solution was washed with water, then dried over $MgSO_4$, and evaporated to give 0.74 g of acidic product, mp 170–185°. The ether solutions containing the neutral products on drying and evaporation gave 1.57 g of semicrystalline material which was dissolved in 15 ml of anhydrous tetrahydrofuran and this solution was added dropwise to 0.20 g of lithium aluminum hydride in 10 ml of tetrahydrofuran. After refluxing for 4 hr, the tetrahydrofuran solution was stirred at room temperature for 12 hr, wet ether was then added, followed by a 20% aqueous NH_4Cl solution, and finally the entire solution was extracted with ether. After drying and evaporation 1.55 g of semicrystalline material was isolated from the ether solution. This material was oxidized with chromic anhydride (2.5 g) in pyridine as described above to give 0.24 g of acidic product.

The combined acid fractions were recrystallized twice from methanol to give 0.76 g of pure XVII: mp 203–205°; λ_{max}^{KBr} 3.0–4.0, 5.90, 14.1, 14.5 μ.

Anal. Calcd for $C_{19}H_{28}O_2$: C, 79.12; H, 9.78. Found: C, 79.30; H, 9.70.

Preparation of XXI.—Thionyl chloride (2.5 ml) was added to a solution prepared by adding 4 drops of pyridine and 0.76 g of XVII to 25 ml of dry ether. After standing at room temperature for 3 hr, the pyridine hydrochloride was removed by filtration and washed with dry ether. The combined ether filtrates were evaporated to give 0.80 g of crude XIX, mp 95–102°, which was directly used without further purification. Acid chloride XIX (0.80 g) in 50 ml of dry ether was added to 10 ml of ethanol containing 3 ml of 95% hydrazine at 0°. The mixture was vigorously stirred for 5 min, then poured into 150 ml of water and extracted with ether. After washing with water and drying ($MgSO_4$), the ether extract was evaporated to give 0.726 g of XX, mp 180–186°. Recrystallization from ethyl acetate gave the analytical sample: mp 185–187°; λ_{max}^{KBr} 2.97, 3.15, 6.15, 10.10, 14.6 μ; nmr ($CDCl_3$) δ 0.87 (s, 3), 1.20 (s, 3), 3.85 (s, broad), 6.02 and 6.10 (these correspond to two lines of the AB part of an ABX system but, since the other lines are not visible a first-order analysis is not possible.)

Anal. Calcd for $C_{19}H_{30}NO_2$: C, 75.45; H, 10.00. Found: C, 74.94, H, 9.95.

The acid hydrazide XX (0.87 g) was dissolved in 28 ml of acetic acid and after cooling to 0°, a saturated solution of 0.69 g of sodium nitrite in water was added and the mixture was vigorously shaken for 3 min. After dilution with ice–water, the solution was exhaustively extracted with *n*-hexane, which in turn washed with ice–water and 5% $NaHCO_3$ and finally dried ($MgSO_4$). The *n*-hexane solution (~480 ml) of XVIII was irradiated in a nitrogen atmosphere, by immersion of a 200-w Hanovia lamp in a quartz tube into the solution, at 15–20° for 10 hr. After filtration, to remove a small amount of insoluble material, the hexane solution was evaporated at room temperature with a rotary evaporator to give 0.79 g of partially crystalline material which was chromatographed on 11 g of Merck acid-washed alumina (activity III). The chloroform–benzene (1:3) eluent yielded 0.16 g of lactam XXI: mp 242–245°; λ_{max}^{KBr} 3.12, 6.05, 14.3 μ; nmr ($CDCl_3$) δ 1.13 (s, 3), 3.38 (q, 2, J = 12 cps), 6.08 and 6.14 (see discussion of nmr of XX).

Anal. Calcd for $C_{19}H_{27}NO$: C, 79.95; H, 9.54. Found: C, 79.64; H, 9.49.

Two other products were obtained in small amounts from the chromatography column, but these were shown not to be the desired lactam XXI by the presence of two methyl groups in each of their nmr spectra.

Registry No.—VIII, 10034-00-1; IX, 10034-01-2; XI, 10034-02-3; XII, 10034-03-4; XIII, 10060-18-1; XIV, 10034-04-5; XV, 734-02-1; XVII, 10034-06-7; XX, 10034-07-8; XXI, 10034-08-9.

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Terpenes. XX. The Synthesis of Postulated Tetracarbocyclic Diterpenoids[1]

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In 1955[3] Wenkert first postulated the biogenesis of tetracarbocyclic diterpenes and diterpenoid alkaloids from pimarene-type precursors *via* bridged carbonium ions. Since this postulation a number of new tetracarbocyclic diterpenes (atisirene,[4] hibaene,[5] kaurene[6]) and one pentacarbocyclic diterpene (trachylobane[7]) have been found in nature, the biogenesis of which are readily accommodated by Wenkert's postulation.

Extension of Wenkert's postulation to include precursors of the isopimarene type would lead to the tetra- and pentacarbocyclic diterpenes shown in Scheme I,[8,9] and indeed *in vitro* syntheses of isohibaene and isohibane from isopimaradiene precursors have been accomplished.[10]

Of this group, only diterpenoids possessing the phyllocladene skelton have thus far been encountered in nature; this may be because the cyclization depicted in Scheme I must involve ring C in a nonchair conforma-

(1) Terpenes XX. Terpenes XIX: L. H. Zalkow, N. N. Girotra, and V. B. Zalkow, *J. Org. Chem.*, **32**, 806 (1967).

(2) Department of Chemistry, Simon Frazer University, Burnaby 2, British Columbia, Canada.

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(6) L. H. Briggs, B. F. Cain, B. R. Davis, and J. K. Wilmhurst, *Tetrahedron Letters*, 8 (1959); C. Djerassi, *et al.*, *J. Am. Chem. Soc.*, **83**, 3720 (1961).

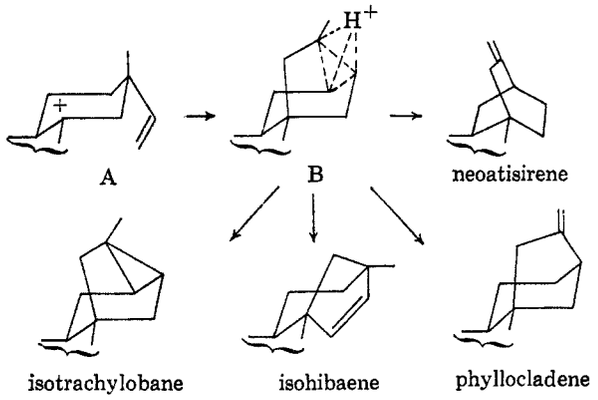
(7) G. Ourisson, *et al.*, *Bull. Soc. Chim. France*, 2882, 2888, 2894, 2903 (1965).

(8) We do not wish to enter into the controversy regarding classical and nonclassical carbonium ions and simply wish to indicate that the isopimarene carbonium ion generated by protonation of the Δ^8 double bond (A) can give rise to the various diterpenoids *via* some intermediate such as B.

(9) Only relative configurations are indicated in Scheme I.

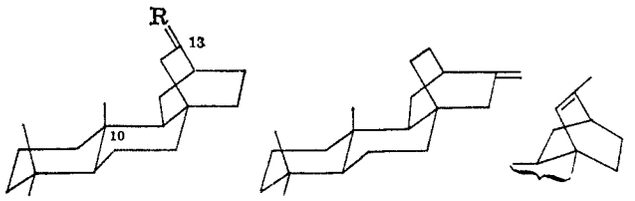
(10) (a) E. Wenkert, P. W. Jeffs, and J. R. Mahajan, *J. Am. Chem. Soc.*, **86**, 2218 (1964); (b) W. Herz, D. Melchior, R. N. Mirrington, and P. J. S. Pauwels, *J. Org. Chem.*, **30**, 1873 (1965). For a similar synthesis of hibaene, see W. Herz, A. K. Pinder and R. N. Mirrington, *ibid.*, **31**, 2257 (1966).

SCHEME I



tion.[11] We wish to record here the synthesis of neoatisirene and its endocyclic isomer, isoneoatisirene.[12]

Ketone I, synthesized as previously described[13] in 13 steps from maleopimaric acid, on treatment with methylenetriphenylphosphorane gave neoatisirene (II), mp 75–76°, $[\alpha]D$ +88.7° [(+)-atisirene (III) synthesized from maleopimaric acid[13] showed mp 60–61°, $[\alpha]D$ +40.1°] after chromatography over alumina. The infrared spectrum of II showed a strong band at



I, R = O
II, R = CH_2

III IV

873 cm^{-1} for the *exo*-methylene group while the nmr spectrum showed one quaternary methyl group at δ 0.81 and two quaternary methyl groups at 0.85. The C-10 methyl group in II shows slight shielding by the $\Delta^{13(17)}$ double bond compared with the C-10 methyl group in III. As observed for III, the olefinic protons in II appear as broad singlets centered at δ 4.51 and 4.70.

Refluxing in glacial acetic acid converted II into neoisoatisirene (IV), mp 76.5–77.5°, $[\alpha]D$ +40.56° [lit.[4] for (−)-isoatisirene mp 84–85°, $[\alpha]D$ −73.99°]. The infrared spectrum of IV showed strong absorption at 829, 813, and 805 cm^{-1} characteristic of trisubstituted double bonds while the nmr spectrum showed methyl singlets at δ 0.51, 0.82, and 0.87. The high-field singlet arises from the C-10 methyl group which is highly shielded by the Δ^{13} double bond.[14] In addition, the methyl group at C-13 appeared as a doublet (J = 1.5 cps) centered at δ 1.90 and the C-14 vinylic proton appeared as a broad signal centered at 5.50. Neoisoatisirene was also prepared directly from ketone I by treatment with methyl Grignard reagent followed by subjecting the resulting alcoholic mixture to dehydration by iodine in refluxing benzene.

(11) We wish to thank the referee for bringing this point to our attention.

(12) The nomenclature chosen in naming neoatisirene and isoneoatisirene is consistent with that already used for the isomeric compounds by Dev, *et al.*,[4] and illustrates the close relationship of the compounds in question.

(13) L. H. Zalkow and N. N. Girotra, *J. Org. Chem.*, **29**, 1299 (1964).

(14) N. N. Girotra and L. H. Zalkow, *Tetrahedron*, **21**, 101 (1965).

Richards and Hendrickson[15] have stated that a structure such as II could easily give rise to isophyllocladene by an acid-catalyzed, *in vitro* isomerization. As seen above this was not observed with acetic acid and treatment of II with 88% formic acid, conditions previously reported[16] to convert rimuene to isophyllocladene, gave a mixture of formate esters and alcohols and a negligible amount of olefinic products.

Experimental Section

Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Analyses were performed by Midwest Microlab, Inc., Indianapolis, Ind. Infrared spectra were recorded using a Beckman IR-5 spectrophotometer. Nmr spectra were recorded with a Varian A-60 nmr spectrometer using TMS as an internal standard (δ = 0).

Synthesis of Neoatisirene.—Methyltriphenylphosphonium bromide (0.96 g, prepared from methyl bromide and triphenylphosphine) was added to 6 ml of 0.40 *N* *n*-butyllithium (obtained by the addition of 10 g of *n*-butyl bromide to 1 g of lithium in 20 ml of ether and titrated just before use) under an oxygen-free nitrogen atmosphere. The mixture was stirred overnight. A solution of 0.45 g of I[13] in 10 ml of anhydrous ether was added to the mixture and stirring was continued for an additional 2 hr. Ether was distilled and anhydrous tetrahydrofuran was added, until most of the ether had been displaced. The reaction mixture was refluxed for 8 hr, then cooled. The mixture was diluted with 200 ml of water and extracted with ether. The organic layer was washed with water and dried over anhydrous magnesium sulfate; evaporation of the solvent yielded a gummy mass. Elution of this mixture through neutral alumina (100 g) with 100 ml of petroleum ether (bp 60–80°) followed by recrystallization from methanol yielded 0.250 g of olefin II, mp 75–76°. Continued elution with 300 ml of petroleum ether–ethyl ether (9:1) gave 0.108 of unreacted ketone, mp 120–121° (lit.[13] mp 125–126°). The analytical sample of II was obtained by recrystallization from methanol and had mp 75–76°; ν_{max}^{KBr} 3000, 1640, 873 cm^{-1}; nmr (CCl_4) δ 0.81 (3), 0.85 (6), 4.51 (broad, one proton) and 4.70 (broad, one proton); $[\alpha]D$ +88.7° ($CHCl_3$).

Anal. Calcd for $C_{20}H_{32}$: C, 88.16; H, 11.83. Found: C, 88.25; H, 12.06.

Acetic Acid Isomerization of II to IV.—A solution of 0.065 g of II in 1.5 ml of glacial acetic acid was refluxed for 6 hr.[17] Evaporation of the acetic acid *in vacuo* left an oil which thin layer chromatography (10 cm) on silica gel G in petroleum ether (developed in iodine vapor) showed to possess one component with R_f 0.71. The starting material II had R_f 0.67 under identical conditions. Gas chromatography of the crude reaction product on a 10 ft × 0.25 in., 10% silicone rubber column at 200° using helium flow rate of 90 cc/min gave one peak with a retention time of 4.25 min. Gas chromatography on a 10 ft × 0.25 in. silicone nitrile column at 200° using a helium flow rate of 150 cc/min showed one component with a retention time of 6.5 min. The crude product crystallized from methanol gave mp 76.5–77.5°; ν_{max}^{KBr} 2870, 1640, 829, 813, 805 cm^{-1}; nmr (CCl_4) δ 0.51 (3), 0.82 (3), 0.87 (3), 1.90 (3, doublet, J = 1.5 cps), 2.26 (broad, one proton) and 5.50 (1); $[\alpha]D$ +40.56° ($CHCl_3$).

Anal. Calcd for $C_{20}H_{32}$: C, 88.16; H, 11.83. Found: C, 88.29; H, 11.71.

Preparation of IV from I.—A solution of 0.094 g of I in 10 ml of anhydrous ether was added to a threefold excess of methyl Grignard reagent (prepared by the addition of 0.142 g of methyl iodide to 0.048 g of magnesium in 20 ml of anhydrous ether) and stirred at room temperature for 4 hr. The excess Grignard reagent was destroyed by addition of dilute, aqueous ammonium chloride solution and the aqueous portion of the reaction mixture was extracted several times with ether. The ether extract was dried over anhydrous magnesium sulfate and evaporated to yield

(15) J. H. Richards and J. B. Hendrickson, "The Biosynthesis of Steroids, Terpenes and Acetogenins," W. A. Benjamin, Inc., New York, N. Y., 1964, pp 249–251.

(16) Recent workers have been unable to confirm the previously reported conversation of rimuene to isophyllocladene: J. D. Connolly, R. McCrindle, R. D. H. Murray, and K. H. Overton, *Tetrahedron Letters*, 1983 (1964).

(17) L. H. Briggs, R. W. Cowley, J. A. Loe, and W. I. Taylor, *J. Chem. Soc.*, 955 (1950).

0.087 g of a gummy mass. The infrared spectrum of the crude product showed weak carbonyl absorption.

Elution of this mixture through neutral alumina (10 g) with petroleum ether–ethyl ether (9:1) gave 0.012 g of II. Elution with petroleum ether–ethyl ether (8:2) gave 0.067 g of a mixture of two alcohols. Thin layer chromatography (10 cm) of the alcoholic fraction on silica gel G in benzene–ethyl acetate (8:2) showed two components with R_f 0.40 and 0.50 (developed in iodine vapor).

The mixture of alcohols from above (0.067 g) was refluxed in 3 ml of benzene containing a crystal of iodine for 8 hr. The benzene was evaporated *in vacuo* and the reaction mixture was chromatographed on neutral alumina. Elution with petroleum ether gave 0.060 g of IV which crystallized from methanol and had mp 76.5–77.5°. A mixture melting point of IV obtained in this manner and that obtained as described above was undepressed. The infrared spectra of samples of IV prepared by these different routes were identical.

Registry No.—II, 7721-78-0; IV, 7721-79-1.

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Studies on the C-20 Epimers of 20-Hydroxycholesterol[1]

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The evidence presently available[2-4] supports the contention that the biosynthetic pathways through which the steroid hormones are derived from cholesterol involve, at the start of the sequence, 20α-hydroxycholesterol (1,Δ^5-cholestene-3β,20α-diol). Thus, specific hydroxylases present in endocrine tissues catalyze the introduction of a hydroxyl group at C-20 of cholesterol in the α configuration. This paper describes some chemical and spectral (infrared and nmr) characteristics of I and also reports the synthesis and properties of its 20β epimer (V, Δ^5-cholestene-3β,20β-diol).[5]

The 20α epimer was first synthesized by Petrow and Stuart-Webb[6] by the condensation of pregnenolone acetate and isohexylmagnesium bromide. From the Cram rule,[7] the expected product of this reaction is 20α-hydroxycholesterol (for the stereochemical designation, see ref 8 and 9). In our hands, this compound was the only product formed during the condensation, although a careful search was made to isolate the 20β epimer.

The synthesis of 20β-hydroxycholesterol (V) was accomplished using Δ^5-etiocholenic acid chloride as starting material. The acid chloride was treated with diisohexylcadmium, as described by Kurath and Capezzuto[10] to give 21-nor-20-ketocholesterol 3-acetate which was then condensed with methylmagnesium bromide. Preliminary purification by chromatography of the reaction mixture gave evidence of two compounds, in a ratio 10:1, which were best separated by fractional crystallization. The minor compound was identified by its infrared spectrum and melting point as 20α-hydroxycholesterol 3β-acetate (II). Although the infrared spectrum of the major component was similar to that of II, the melting point of the second product (VI) was much lower than that of II. Prediction from the Cram rule leads to the presumption that VI is 3β-acetoxy-Δ^5-cholesten-20β-ol. Saponification yielded V, the infrared spectrum of which is very like that of I. Each of the alcohols, their acetates, benzoates and the Δ^4-3-ketones derived from the alcohols have distinctive melting points (Table I). However, only mixtures of the isomeric alcohols and of their derived Δ^4-3-ketones gave true mixture melting point depressions. In the case of the acetates and the benzoates, the melting points of mixtures of the isomers were unsharp and occurred between those of the individual isomers. The MD values for the isomeric alcohols, their acetates, and benzoates were found to be almost identical for each pair. This is in contrast to the isomeric 22-hydroxycholesterols[11] and the 24-hydroxycholesterols[12] in which the β isomers were found to be more levorotatory than are the α epimers. The rotations of the isomeric Δ^4-3-ketocholesten-20-ols appear to differ significantly.

TABLE I

PHYSICAL CONSTANTS OF 20α- AND 20β-HYDROXYCHOLESTEROLS AND SOME OF THEIR DERIVATIVES

Substituents		20α-Hydroxy series			20β-Hydroxy series		
C_3	C_5	No.	mp, °C	$[\alpha]_D$, deg	No.	mp	$[\alpha]_D$, deg
β-OH	Δ^5	I	136–137	−57	V	115–117	−61
β-OAc	Δ^5	II	156–157	−59	VI	113–114	−47
β-OBz	Δ^5	III	176–178	−38	VII	144–145	−34
Keto	Δ^4	IV	135–137	+65	VIII	129–130	+77

Determination of the infrared spectra of the compounds described in this study revealed that each member of an isomeric pair possesses virtually the same infrared spectrum as its epimer. Moreover, a great similarity was found between the spectrum of cholesterol and those of both 20-OH cholesterols. Thus, the use of infrared spectra as criteria for identification of compounds of this series, at least, is unreliable.

In contrast to the infrared spectra, the nmr spectra of the isomeric 20-hydroxycholesterols and their acetates show very distinct differences in the chemical shifts of the methyl proton resonances. The protons of special interest in this study are the methyl protons on C-18, C-19, C-21, C-26, and C-27. Firm spectral assignments for these protons have been presented[13] for cholesterol. On the basis of these assignments and the relative proton counts under the various peaks, assignment of the features of the spectra of I and V was

(1) This work was supported in part by funds from U. S. Public Health Service Grant No. 00110.

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(5) Some of the findings reported in this paper have been presented. See L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p 344.

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(8) P. A. Platner, *Helv. Chim. Acta*, **34**, 1693 (1951).

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(11) K. Tsuda and R. Hayatsu, *ibid.*, **81**, 5987 (1959).

(12) N. Klyne and W. N. Stokes, *J. Chem. Soc.*, 1979 (1954).

(13) "High Resolution N. M. R. Spectra Catalog," Vol. 1, Varian Associates, Inc., Palo Alto, Calif., 1962, Spectrum No. 363.