Tetrahedron 1963, Vol. 19, pp. 1101 to 1107. Pergamon Press Ltd. Printed in Northern Ireland

CONSTITUTION AND ABSOLUTE CONFIGURATION OF EREMOPHILENOLIDE

L. NOVOTNÝ, J. JIZBA, V. HEROUT and F. ŠORM[1]
Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science

L. H. ZALKOW[2] and S. HU
Department of Chemistry, Oklahoma State University

CARL DJERASSI[3]
Department of Chemistry, Stanford University

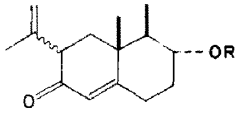
(*Received* 12 *December* 1962)

Abstract—Eremophilenolide, a naturally occurring sesquiterpenoid from *Petasites hybridus*, has been shown to be based on a *cis*-fused decalin system (IV) by multistep degradation to the *cis*-β-decalone (XIV), which could also be obtained from hydroxyeremophilone (XV). Since the absolute configuration of the latter is known, the present interconversion settles the absolute configuration of eremophilenolide as well as that of the other sesquiterpenes with which it has previously been inter-related.

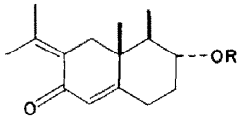
IN recent years there has been described the isolation,[4–6] structure proof[7,8] and establishment of absolute configuration[9,10] of petasin (I), isopetasin (II) and S-petasin (III). These three sesquiterpenoid constituents of *Petasites hybridus* (L.) Fl. Wett. (syn. *P. officinalis* Moench.) are based on the rare eremophilane skeleton (e.g. VIII) which does not follow the classical isoprene rule, although its biogenesis is readily accommodated[11] by methyl migration from an eudalenoid precursor. *Petasites officinalis* Moench. of Czechoslovak origin does not contain petasin (I) and its congeners, but rather a series of novel sesquiterpenes[12–15] of the eremophilane type with additional furan or α,β-unsaturated-γ-lactone groupings. One of these is the lactone eremophilenolide for which we now report the structure and absolute configuration IV.[16] Since this substance has already been related[12] to the other novel

[1] Paper CL in the series "On Terpenes" from the Czechoslovak Academy of Science. For preceding article see J. Vrkoč, V. Herout, F. Šorm, *Coll. Czech. Chem. Commun.*, **28**, 1084 (1963).
[2] Paper IV in the series "Terpenes" from Oklahoma State University. For preceding article see L. H. Zalkow, V. B. Zalkow and D. R. Brannon, *Chemistry & Industry*, **38** (1963).
[3] Paper LII in the series "Terpenoids" from Stanford University. For preceding article see C. Djerassi and R. McCrindle, *J. Chem. Soc.*, 4034 (1962).
[4] A. Stoll, R. Morf, A. Rheiner and J. Renz, *Experientia*, **12**, 360 (1956).
[5] A. Aebi, J. Büchi, T. Waaler, E. Eichenberger and J. Schmutz, *Pharm. Acta Helv.*, **30**, 277 (1955).
[6] A. Aebi, T. Waaler and J. Büchi, *Pharm. Weekblad*, **93**, 397 (1958).
[7] T. Waaler, Thesis, E.T.H., Zurich, Juris Verlag, 1957.
[8] A. Aebi and T. Waaler, "Über die Inhaltsstoffe von *Petasites hybridus* (L.) Fl. Wett., Verlag Helbing und Lichtenhahn, Basel, 1959.
[9] A. Aebi and C. Djerassi, *Helv. Chim. Acta*, **42**, 1785 (1959).
[10] D. Herbst and C. Djerassi, *J. Amer. Chem. Soc.*, 82, 4337 (1960).
[11] R. Robinson, "The Structural Relations of Natural Products", Oxford University Press, 1955, p. 12. See also J. B. Hendrickson, *Tetrahedron*, **7**, 82 (1959).
[12] L. Novotný, V. Herout and F. Šorm, *Tetrahedron Letters*, 697 (1961).
[13] L. Novotný, J. Jizba, V. Herout and F. Šorm, *Coll. Czech. Chem. Commun.*, **27**, 1393 (1962).
[14] L. Novotný, V. Herout and F. Šorm, *Coll. Czech. Chem. Commun.*, **27**, 1400 (1962).
[15] J. Hochmannová, L. Novotný and V. Herout, *Coll. Czech. Chem. Commun.*, **27**, 1870 (1962).

sesquiterpenoid constituents of this plant, the present absolute configurational assignments apply *ipso facto* to them.



I R = -COC(CH$_3$) = CHCH$_3$
III R = -COCH = CHSCH$_3$



II R = -COC(CH$_3$) = CHCH$_3$

Eremophilenolide ($C_{15}H_{22}O_2$) exhibits I.R. bands at 1760 and 1693 cm^{-1} typical of an α,β-unsaturated 5-membered lactone and the U.V. absorption spectrum ($\log \varepsilon_{max}^{220-224}$ 4·16) was compatible with such a chromophore. Confirmation was adduced by catalytic hydrogenation (acetic acid–platinum oxide) to dihydroeremophilenolide (V), the I.R. spectrum (1780 cm^{-1}) of which was now characteristic of a saturated γ-lactone. The carbon skeleton of eremophilenolide was established by the following reaction sequence:

Lithium aluminium hydride reduction of dihydroeremophilenolide (V) afforded the saturated diol VI, which was converted to the crystalline ditosylate. Treatment with lithium aluminium hydride gave a mixture consisting of a hydrocarbon ($C_{15}H_{26}$) and an ether ($C_{15}H_{26}O$). The hydrocarbon was unsaturated (VII) and upon catalytic hydrogenation provided the saturated liquid hydrocarbon VIII, the infrared spectrum of which was identical with eremophilane obtained earlier[15] from hydroxydihydroeremophilone (XVI). The other liquid constituent ($C_{15}H_{26}O$) of the lithium aluminium hydride reduction of the ditosylate of VI exhibited an I.R. spectrum identical with that of tetrahydrofuranoeremophilane (IX), the principal catalytic hydrogenation product[15] of the naturally occurring furanoeremophilane ($\bar{x}$). There remains only the question of the termination point of the lactone ring (C-6 or C-8)[17] and this was resolved in favor of C-8. Thus when the lithium aluminium hydride reduction of dihydroeremophilenolide (V) was performed under controlled conditions[18] and the intermediate hydroxyaldehyde XI immediately subjected to Huang-Minlon reduction,[19] there was isolated the crystalline hydroxyeremophilane (XII). Oxidation of the latter with chromium trioxide in acetone solution[20] provided the ketone XIII, characterized as the semicarbazone m.p. 161–164°, which could be isomerized with base to the ketone XIV, forming a higher melting semicarbazone (m.p. 196–198°). The I.R. spectrum of the unstable ketone exhibited a band at 1430 cm^{-1}, suggestive of a methylene group adjacent to a ketonic function (1711 cm^{-1}), an observation which pointed towards C-8 as the termination point of the lactone ring. Full confirmation for this structural supposition as well as evidence bearing on the stereochemistry of the ketones XIII and XIV was obtained in the following manner.

In the original proof of absolute configuration[21] of eremophilone, the methyl

[16] All structures in the present article imply absolute configuration assignments utilizing the conventional steroid notation.

[17] The numbering system (see IV) is based on that of the presumed eudalenoid biogenetic precursor.

[18] B. C. Bhattacharyya, A. S. Rao and M. Shaligram, *Chemistry & Industry*, 469 (1960).

[19] Huang-Minlon, *J. Amer. Chem. Soc.*, **68**, 2487 (1946).

[20] K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.* 39 (1946).

[21] L. H. Zalkow, F. X. Markley and C. Djerassi, *J. Amer. Chem. Soc.*, **81**, 2914 (1959); *ibid.*, **82**, 6354 (1960).

ether (XVc) of hydroxyeremophilone was hydrogenated and after base equilibration at C-7, the methoxy function was removed with calcium in liquid ammonia and the intermediate C-8 hydroxyl group re-oxidized. The resulting ketone XVII exhibited a positive Cotton effect, typical[22] of A/B *trans*-fused 3-keto steroids, and proved to be identical with a synthetic specimen of known constitution and absolute configuration. During a recent repetition of this sequence, it was possible to isolate from the mother liquors of the 2,4-dinitrophenylhydrazone (m.p. 170–172°) of the *trans* ketone XVII a small amount of an isomeric dinitrophenylhydrazone (m.p. 169–170°), which did not give any melting point depression upon admixture with the 2,4-dinitrophenylhydrazone (m.p. 170–172°) derived from the base-equilibrated ketone XIV arising from the above described dihydroeremophilenolide (V) degradation. This latter ketone exhibited an optical rotatory dispersion curve characteristic[22] of A/B *cis*-fused 3-keto steroids indicating that in the catalytic hydrogenation[21] of hydroxyeremophilone methyl ether (XVc) there is produced a small quantity of the *cis* isomer in addition to the predominant *trans* ketone XVII.

In order to put this interconversion of eremophilenolide (IV) with hydroxyeremophilone (XVa) on a firm footing, attempts were made to increase the proportion of *cis*-fused hydrogenation product. Indeed, when the catalytic hydrogenation was performed with hydroxyeremophilone (XVa) itself, there was obtained an oily tetrahydro derivative (XVIIIa), the optical rotatory dispersion curve of which indicated the presence of substantial amounts of *cis*-fused isomer. Acetylation provided a mixture of tetrahydrohydroxyeremophilone acetate isomers (XVIIIb), the infrared spectrum and optical rotatory dispersion curve of which were virtually identical with those of the direct hydrogenation product of hydroxyeremophilone acetate (XVb). Deacetoxylation with calcium in liquid ammonia[23] and re-oxydation of over-reduced ketone furnished an approximate 1:1 mixture of the *cis* (XIV) and *trans* (XVII) ketones, which could be separated by fractional crystallization of their 2,4-dinitrophenylhydrazones and semicarbazones. The melting points of these two derivatives of the *cis*-ketone XIV proved to be identical with those of the specimens originating from eremophilenolide (IV) and the optical rotatory dispersion curves exhibited the typical negative Cotton effect, superimposed upon a positive background, as is so characteristic[22] of A/B *cis* fused 3-keto steroids.

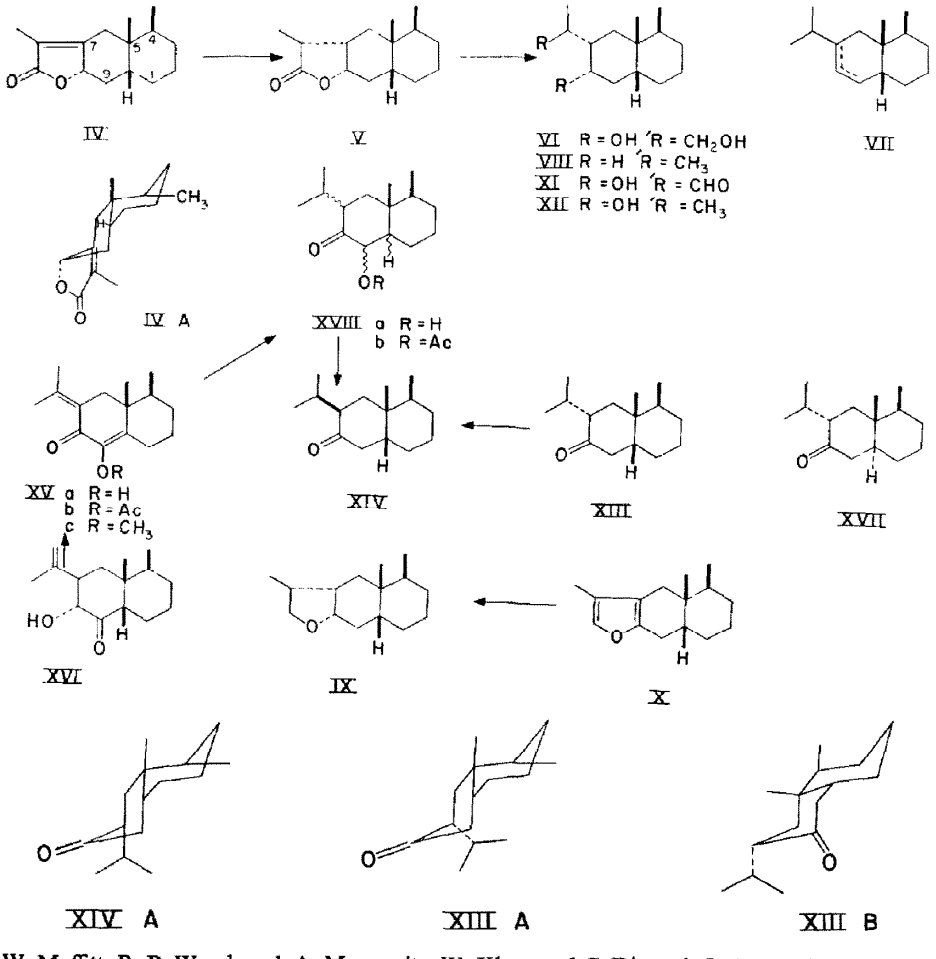
This interconversion of eremophilenolide (IV) and hydroxyeremophilone (XVa) completely settles the structure and absolute configuration of the former. Furthermore, the isolation of a base-labile (XIII) and a base-stable (XIV) *cis*-fused ketone permits unequivocal stereochemical assignment to C-7. Catalytic hydrogenation of a *cis*-octalin system (e.g. VII with 7-8 double bond or exocyclic double bond in IV) would be expected to occur predominantly from the less hindered β-side,[16] thus giving rise to the unstable ketone XIII, which could exist in either the "steroid" conformation XIIIA or the "non-steroid" conformation XIIIB (or in some intermediate distorted conformation). Either one would obviously be less favored than "steroid" conformation XIVA of the base-stable *cis* ketone and it should be noted that the negative

[22] C. Djerassi, "Optical Rotatory Dispersion: Applications to Organic Chemistry", McGraw-Hill Book Co., New York, 1960.

[23] J. H. Chapman, J. Elks, G. H. Phillips and L. J. Wyman, *J. Chem. Soc.*, 4344 (1956). See also J. S. Mills, H. J. Ringold and C. Djerassi, *J. Amer. Chem. Soc.*, **80**, 6118 (1958).

Cotton effect (see Experimental) of XIV is consistent, according to the octant rule,[24] with this conformational assignment. As indicated below, the *cis* ring fusion in eremophilenolide (IV) points towards the α-orientation of the C-8 oxygen atom.

The presence of the C-4 equatorial methyl group makes the "steroid-like" conformation (e.g. XIIIA or XIVA) of the decalin system clearly preferred over the "non-steroid" conformation (e.g. XIIIB). In the "steroid-like" conformation, the lactone ring in IV can only be formed with a hydroxyl group at C-8, which is α-oriented in a chair cyclohexane ring. A β-connection at C-8 would require that ring to exist in a very unfavorable boat form. While this is *a priori* not impossible in a natural product, application of the modified Klyne-Hudson[25] rule using the molecular rotation values of −12° (V) and +42° (IX) leads to an 8α (R)[26] stereochemical assignment and hence to the stable all-chair "steroid-like" conformation IVA. Catalytic hydrogenation of such a double bond should occur principally from the



[24] W. Moffitt, R. B. Woodward, A. Moscowitz, W. Klyne and C. Djerassi, *J. Amer. Chem. Soc.*, **83**, 4013 (1961).

[25] V. Sýkora and M. Romaňuk, *Coll. Czech. Chem. Commun.*, 22, 1909 (1957).

[26] R. S. Cahn, C. K. Ingold and V. Prelog, *Experientia*, 12, 82 (1956).

unhindered β-face, thus leading to the α-orientation at C-7 (e.g. V, VI, IX, etc.) and hence to a base-labile ketone XIII.

EXPERIMENTAL

All m.p.'s were determined on the Kofler block. The rotatory dispersion curves were measured by Mrs. Ruth Records on a Nippon Bunko (Japan Spectroscopic Manufacturing Co.) automatically recording spectropolarimeter model ORD-2.

Dihydroeremophilenolide (V)[13]

Eremophilenolide (IV; 5·0 g) was hydrogenated at room temp and atm. press. over a period of 50 hr in acetic acid solution in the presence of 0·5 g platinum oxide catalyst. The product was purified by chromatography on neutral alumina (activity IV) and elution with pet ether. Recrystallization from pentane afforded 3·63 g of colorless crystals, m.p. 73–73·5°, $[\alpha]_D^{20}$ −5° (*c*, 5·86 in $CHCl_3$) (Found: C, 76·08; H, 10·13. Calc. for $C_{15}H_{24}O_2$: C, 76·22; H, 10·24%).

Eremophilan-8,12-*diol* (VI)

Dihydroeremophilenolide (V; 3·77 g) was reduced with excess (5·0 g) lithium aluminium hydride in ether solution by heating under reflux for 5 hr. After decomposition with saturated sodium sulfate solution, the ether solution was washed, dried and evaporated to afford, after distillation 3·92 g diol VI as a colorless oil, b.p. 135°/0·02 mm (Found: C, 74·78; H, 11·44; active hydrogen 0·78. Calc. for $C_{15}H_{28}O_2$: C, 74·95; H, 11·74%; active hydrogen, 0·84).

Treatment of the diol VI (3·8 g) with *p*-toluenesulfonyl chloride in pyridine solution at 0° for 48 hr gave after recrystallization from ether–light petroleum 1·88 g *ditosylate* in two polymorphic forms, m.p. 75–76° and 85–86° (Found: C, 63·19; H, 7·02; S, 11·79. Calc. for $C_{29}H_{40}O_6S_2$: C, 63·47; H, 7·35; S, 11·68%).

Reduction of eremophilan-8,12-*diol* (VI) *ditosylate with lithium aluminum hydride*

The preceding ditosylate (1·88 g) was reduced with 1·2 g lithium aluminum hydride in boiling ether for 2 hr. The reaction mixture was decomposed with saturated sodium sulfate solution and the crude product separated by chromatography on alumina (activity III). The first light pet. ether eluates contained the unsaturated hydrocarbon *eremophil*-7(or 8)-*ene* (VII; 300 mg), which was redistilled *in vacuo* before analysis and which exhibited an I.R. band (neat) of weak intensity at 1669 cm^{-1}. (Found: C, 87·09; H, 12·76. Calc. for $C_{15}H_{26}$: C, 87·30; H, 12·70%).

Further elution with light pet ether afforded 410 mg *tetrahydrofuranoeremophilane* (IX), b.p. 97·5°/0·1 mm, $[\alpha]_D^{20}$ +19° (neat), the I.R. spectrum of which was identical with that of the hydrogenation product[15] of furanoeremophilane (X) (Found: C, 81·17; H, 11·70. Calc. for $C_{15}H_{26}O$: C, 81·02; H, 11·79%).

Eremophilane (VIII)

Catalytic hydrogenation of 300 mg eremophilene (VII) in acetic acid solution in the presence of platinum oxide catalyst provided after distillation *in vacuo* a colorless oil, d_4^{20} 0·8944, n_D^{20} 1·4848, $[\alpha]_D^{20}$ −18·5° (neat) (Found: C, 86·70; H, 13·54. Calc. for $C_{15}H_{28}$: C, 86·45; H, 13·54%).

Eremophilan-8-*ol* (XII)

To a stirred solution of 2·25 g dihydroeremophilenolide (V) in 20 cc dry dioxane was added at −15° over a period of 20 min 4·5 cc ethereal solution of lithium aluminum hydride (1 cc = 19·63 mg of reagent). After 1 hr, the temp of the reaction mixture had reached 20° at which time 5N sulfuric acid was added and the product isolated in the usual manner. The total crude hydroxy aldehyde XI was heated for 4 hr at 195–200° with 2·5 g 75% hydrazine hydrate, 2·5 g sodium hydroxide and 10 cc ethylene glycol. The cooled mixture was acidified with tartaric acid and the crude product (2·8 g, isolated by extraction with ether) was chromatographed on 200 g activity IV alumina. The desired eremophilanol XII (0·5 g) was eluted with 1:1 benzene–light petroleum and exhibited m.p. 59–59·5° after recrystallization from aqueous ethanol. Its I.R. spectrum (chloroform solution) exhibited a band at 3624 cm^{-1} but no carbonyl absorption (Found: C, 79·92; H, 12·73. Calc. for $C_{15}H_{28}O$: C, 80·29; H, 12·58%).

(7α)-*Eremophilan*-8-*one* (XIII)

Oxidation of 0·71 g eremophilanol (XII) was effected at 20° in acetone solution over a period of 10 min by titration with a standard chromium trioxide solution.[20] The solvent was removed *in vacuo*, the product was extracted with chloroform and the liquid ketone XIII distilled at 97°/0·4 mm; yield, 0·70 g, I.R. carbonyl band (neat) at 1711 cm^{-1}. (Found: C, 80·90; H, 11·45. Calc. for $C_{15}H_{26}O$: C, 81·02; H, 11·79%).

The *semicarbazone* was prepared in methanol solution at 20° (20 hr) by the semicarbazide acetate procedure and the solid recrystallized from ether, whereupon it showed m.p. 161–164° (Found: C, 68·42; H, 10·14; N, 14·96. Calc. for $C_{16}H_{29}N_3O$: C, 68·77; H, 10·46; N, 15·04%).

Preparation of the 2,4-dinitrophenylhydrazone on keeping the ketone XIII at room temperature for several hours in ethanolic solution with 2,4-dinitrophenylhydrazine effected also inversion at C-7 and after recrystallization from methanol there was isolated the 2,4-dinitrophenylhydrazone of the 7β-isomer XIV, m.p. 170·5–172·5° (Found: N, 13·72. Calc. for $C_{21}H_{30}N_4O_4$: N, 13·92%).

(7β)-*Eremophilan*-8-*one* (XIV)

(a) *From* (7α)-*eremophilan*-8-*one* (XIII). The ketone XIII (300 mg) was heated under reflux in a nitrogen atmosphere in methanol solution with a catalytic amount of sodium and the epimerized ketone XIV was extracted with ether and converted directly by the semicarbazide acetate procedure into the *semicarbazone*, which exhibited m.p. 196–198° after recrystallization from ethanol (Found: C, 68·72; H, 10·50; N, 15·23. Calc. for $C_{16}H_{29}N_3O$: C, 68·77; H, 10·46; N, 15·04%).

The free ketone XIV was obtained from the semicarbazone by steam distillation with a saturated oxalic acid solution and after redistillation exhibited the following optical constants: $[\alpha]_D^{20}$ +33° (*c*, 4·27 in $CHCl_3$); R.D. in methanol (*c*, 0·103): $[\alpha]_{589}$ +8°, $[\alpha]_{350-375}$ ~+50°, $[\alpha]_{314}$ −19°, $[\alpha]_{270}$ +404°. The 2,4-*dinitrophenylhydrazone* possessed m.p. 169–172° after recrystallization from ethanol and did not show any m.p. depression upon admixture with the specimen prepared directly from the 7α-epimer XIII.

(b) *From hydroxyeremophilone* (XVa). Hydrogen consumption equivalent to two molar equivalents ceased within 2 hr when 1·0 g hydroxyeremophilone (XVa) was hydrogenated in 20 cc 95% ethanol and 10% palladium–charcoal catalyst (0·2 g) at room temp and atm. press. Filtration of the catalyst, dilution with water, isolation of the product with ether and vacuum distillation provided 0·9 g *tetrahydrohydroxyeremophilone* (XVIIIa) as a colorless oil, b.p. 70°/0·01 mm, which oxidized to the α-diketone on standing in the air; R.D. in methanol (*c*, 0·21 to 310 mμ, then 0·042): $[\alpha]_{589}$ +73°, $[\alpha]_{345-375}$ ~+200° (broad), $[\alpha]_{332\cdot5}$ +107°, $[\alpha]_{285}$ +1870°, $[\alpha]_{250}$ −330°. The I.R. spectrum ($CHCl_3$) exhibited bands at 3450 and 1708 cm^{-1}. (Found: C, 75·56; H, 10·77; O, 13·84. Calc. for $C_{15}H_{26}O_2$: C, 75·58; H, 11·00; O, 13·42%).

Acetylation of XVIIIa was effected in nearly quantitative yield with acetic anhydride and pyridine (42 hr at 5°) to furnish *tetrahydrohydroxyeremophilone acetate* (XVIIIb) as a viscous oil, b.p. 80°/0·05 mm, R.D. in methanol (*c*, 0·08): $[\alpha]_{589}$ +51°, $[\alpha]_{365-375}$ ~+165° $[\alpha]_{335}$ +42°, $[\alpha]_{300}$ +1294°, $[\alpha]_{270}$ +164° (Found: C, 73·29; H, 9·87; O, 17·43. Calc. for $C_{17}H_{28}O_3$: C, 72·82; H, 10·06, O, 17·12%).

Tetrahydrohydroxyeremophilone acetate (XVIIIb; 1·0 g) in 15 cc dioxane was added slowly to a solution of 0·5 g calcium in 70 cc liquid ammonia. The solution was maintained under reflux for 2 hr and the ammonia was then permitted to evaporate at room temp, followed by the addition of 5 cc 95% ethanol and 10 cc saturated aqueous solution of ammonium chloride. Neutralization with dil. hydrochloric acid and ether extraction gave an oil, the I.R. spectrum of which exhibited strong hydroxyl absorption. Consequently, the total product was oxidized in acetone solution[20] at 10° with chromium trioxide and the resulting ketone (700 mg colorless oil, b.p. 100°/0·1 mm) was transformed directly into the 2,4-dinitrophenylhydrazone with a methanolic hydrochloric acid solution of 2,4-dinitrophenylhydrazine. Fractional recrystallization of the crude derivative afforded approximately equal amounts of two dinitrophenylhydrazones.

The less soluble derivative, m.p. 170–172°, proved to be identical by mixed m.p. determination and I.R. comparison with the previously described[21] dinitrophenylhydrazone of the synthetic ketone XVII. For further characterization, the derivative was heated under reflux for 30 min in acetone solution with stannous chloride and hydrochloric acid,[27] followed by addition of 2N sodium hydroxide

[27] N. M. Cullinane and B. F. R. Edwards, *J. Chem. Soc.*, 1311 (1958).

solution and removal of the acetone. Acidification with hydrochloric acid, extraction with ether and distillation provided the pure *trans* ketone XVII, which was shown to be identical by optical rotatory dispersion and infrared spectral comparison with a totally synthetic specimen.[21]

The more soluble 2,4-dinitrophenylhydrazone, though sharp melting (m.p. 158–159°), represented a mixture of the derivatives of the ketones XIV and XVII. Purification was best effected by cleavage[27] of the 2,4-dinitrophenylhydrazone and conversion of the free ketone mixture to the semicarbazone by the semicarbazide acetate method followed by recrystallization from 95% ethanol. In this manner there was obtained the pure *semicarbazone* of (7β)-*eremophilan*-8-*one* (XIV), m.p. 192–194°, undepressed upon admixture with a specimen derived from eremophilenolide (IV) (Found: C, 68·63; H, 10·38. Calc. for $C_{16}H_{29}N_3O$: C, 68·77; H, 10·46%). The semicarbazone of the contaminating *trans* ketone XVII could be recovered from the mother liquors.

A portion (220 mg) of the semicarbazone of XIV was cleaved by heating under reflux for 2 hr with 10 cc 10% hydrochloric acid and the ketone XIV extracted with ether and distilled at 100°/0·1 mm; yield, 140 mg, rotatory dispersion curve of A/B *cis*-fused 3-keto steroid type as detailed above for the sample originating from eremophilenolide (IV) (Found: C, 80·99; H, 11·83. Calc. for $C_{15}H_{26}O$: C, 81·02; H, 11·79%).

Transformation of the ketone to the 2,4-*dinitrophenylhydrazone* and recrystallization from ethanol gave yellow crystals of m.p. 169–170°, which did not depress the m.p. of the 2,4-dinitrophenylhydrazone of the ketone XIV obtained from eremophilenolide (IV), but which exhibited a marked depression (m.p. 150–160°) when mixed with the 2,4-dinitrophenylhydrazone of the *trans* ketone XVII[21] (Found: C, 62·54; H, 7·44. Calc. for $C_{21}H_{30}N_4O_4$: C, 62·66; H, 7·51%).

(c) *From hydroxyeremophilone acetate* (XVb). Hydroxyeremophilone acetate (XVb) was prepared as previously described,[28] and was hydrogenated with 10% palladium–charcoal catalyst in 95% ethanolic solution at room temp and atm. press. The resulting *tetrahydrohydroxyeremophilone acetate* (XVIIIb) exhibited an I.R. spectrum virtually identical with that of the above described sample obtained by acetylation of the hydrogenation product XVIIIa of hydroxyeremophilone (XVa). On treatment with calcium and liquid ammonia followed by reoxidation with chromium trioxide in acetone solution and separation *via* the 2,4-dinitrophenylhydrazones and semicarbazones, approximately equal amounts of the ketones XIV and XVII were isolated.

(d) *From hydroxyeremophilone methyl ether* (XVc). Hydroxyeremophilone methyl ether (XVc) had been converted previously[21] into the *trans* ketone XVII by the following sequence of reactions: (1) hydrogenation; (2) epimerization with base; (3) calcium–ammonia demethoxylation and (4) reoxidation. A careful reinvestigation of this sequence has shown that the crude ketone XVII initially isolated is contaminated with a small amount of the *cis* isomer XIV. When this crude ketone was converted to the semicarbazone, the derivative (m.p. 178–181°) of the predominant product (XVII) precipitated, uncontaminated with the semicarbazone (m.p. 192–194°) of the minor *cis* isomer. However, when the separation was effected through the 2,4-dinitrophenylhydrazones, recrystallization from ethanol provided the earlier described[21] dinitrophenylhydrazone (m.p. 170–172°) of the *trans* ketone XVII, as well as from the mother liquors a small amount of the 2,4-dinitrophenylhydrazone (m.p. 169–170°) of the *cis* isomer XIV. Identity was established in each instance by appropriate mixture melting point comparisons.

Acknowledgments—The work at Oklahoma State University and at Stanford University was supported by the National Institute of Health through grants AM-05490-02 and AM-06840-04. We are deeply indebted to Dr. Maurice D. Sutherland of the University of Queensland for supplies of hydroxyeremophilone and hydroxydihydroeremophilone.

[28] C. Djerassi, R. Mauli and L. H. Zalkow, *J. Amer. Chem. Soc.*, **81**, 3424 (1959).