PHYSICAL PROPERTIES AND ANALYSIS OF EPHEDRINES AND DERIVATIVES

Compound	M.p., °C.	$[\alpha]^{20}D$ (solvent)	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Oxazolidine	39–43 (44–45)[12]	+22.0°(EtOH) (+23.0)[12]						
ψ-Oxazolidine	Oil	+38.0(EtOH)						
Spirodecane	77–77.5	+8.0($CHCl_3$)	78.4	78.5	9.5	9.4	5.7	6.0
ψ-Spirodecane	72–72.5	+41.3($CHCl_3$)	78.4	78.6	9.5	9.3	5.7	5.6
Oxazolidone	91–92	−110.6($CHCl_3$)	69.1	69.3	6.8	7.0	7.3	7.1
ψ-Oxazolidone	50–51 (50–51)[13]	+29.9($CHCl_3$)	69.1	69.3	6.8	7.0	7.3	7.2
(−)-Ephedrine	39.7	−15.4($CHCl_3$)						
(+)-ψ-Ephedrine	117–118	+99.8($CHCl_3$)						

(−)-ephedrine derivatives where the C_N–CH_3 bond eclipses the $C_OC_6H_5$. No real significance can be attached to the identity of the $C_OC_6H_5$ resonance in the spectra of the oxazolidines since the strong chloroform solvent proton signal effectively masked the weaker phenyl proton signal.

Conclusion.—Differences in the n.m.r. spectra of diastereoisomeric pairs of compounds obtained under conditions of identical concentration and solvent can be interpreted in terms of the relative configuration of the asymmetric centers in the molecule. This is especially true when one of the centers contains a group such as phenyl which exerts a marked magnetic anisotropy effect upon adjacent groups.

Preparative

1,2-Dimethyl-3-phenyl-4-oxa-1-aza-cyclopentan-5-one.—The diastereoisomeric pair of oxazolidones were prepared from (−)-ephedrine (anhydrous) and (+)-ephedrine (method of Newman and Kutner[11]): 13 g. of phosgene in 25 ml. of toluene was added to a solution of 5 g. of the ephedrine in 12.5% caustic soda solution. A yield of approximately 80% was obtained in both cases. Recrystallization was from alcohol.

(11) M. S. Newman and A. Kutner, THIS JOURNAL, **73**, 4199 (1951).

1,2,5,5-Tetramethyl-3-phenyl-4-oxa-1-aza-cyclopentane.—Two grams of the corresponding ephedrines were dissolved in 10 ml. of acetone and 25 ml. of benzene and refluxed for 3 days. Solvent then was evaporated and reflux continued for a further 24 hours with fresh acetone. On reducing the bulk of the solution an oily viscous layer separated in both cases. After drying over anhydrous potassium carbonate the oils were vacuum distilled. In the (−)-ephedrine case the distillate solidified on standing to yield oily crystals. Crystallization could not be induced in the (+)-ψ-ephedrine case.

1,2-Dimethyl-3-phenyl-4-oxa-1-aza-spiro(4,5)decane.—Three and five-tenths grams of the corresponding ephedrines was refluxed in 10 ml. of cyclohexanone and 30 ml. of benzene for 7 hours. The theoretical amount of water azeotroped out in each case. The reaction appeared to proceed much more readily than in the case with acetone. Excess cyclohexanone was removed under moderate vacuum (40 mm.) and the product distilled at between 150 and 160° at 8 mm. Both distillates solidified on cooling and were recrystallized from ethanol. Crude yields of 90% were obtained in both cases.

Acknowledgment.—The author expresses his thanks to Dr. Aksel A. Bothner-By for helpful comments and suggestions regarding the interpretation of the spectra.

(12) K. Freudenberg and J. Geiger, *Ann.*, **575**, 145 (1952).
(13) W. J. Close, *J. Org. Chem.*, **15**, 1131 (1950).

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE FLORIDA STATE UNIVERSITY]

Constituents of Helenium Species. VIII. Isolation and Structure of Balduilin[1]

BY WERNER HERZ, R. B. MITRA[2] AND P. JAYARAMAN

RECEIVED MAY 20, 1959

A new sesquiterpene lactone, balduilin, has been isolated from *Balduina uniflora* Nutt. Conversion to derivatives of helenalin and tenulin establishes its gross structure as I or II (R = Ac). The new guaianolide is a C_6-epimer of the tenulin series and a C_8-epimer of the helenalin series of compounds. Helenalin and tenulin are therefore of opposite absolute configuration at C_6 and C_8, but not at C_7.

In an earlier paper[3] the isolation of helenalin[4] from *Balduina angustifolia* (Pursh) Robins.[5] was reported. Since the genus *Balduina* is technically placed in a tribe of *Compositae* different from *Heleniae* (the tribe containing the genus *Helenium*) further examination of *Balduina* species was expected to yield results of chemical and taxonomic interest. The findings reported in this paper fully substantiate this expectation.

(1) Previous paper, W. Herz, R. B. Mitra, K. Rabindran and W. A. Rohde, THIS JOURNAL, **81**, 1481 (1959).
(2) Recipient of a Fulbright Travel Award, 1957–1958.
(3) W. Herz and R. B. Mitra, THIS JOURNAL, **80**, 4876 (1958).
(4) R. Adams and W. Herz, *ibid.*, **71**, 2346, 2551, 2554 (1949); G. Büchi and D. Rosenthal, *ibid.*, **78**, 3860 (1956).
(5) This species is also referred to as *Actinospermum angustifolium* (Pursh) T and G.

Extraction of *Balduina uniflora* Nutt., a late summer and fall-flowering composite found in the low pinelands of the Southern coastal plain, gave in 0.15% yield a new sesquiterpene lactone $C_{17}H_{20}O_5$, m.p. 231–232°, $[\alpha]^{24}D$ 57°, which we have named balduilin.

Balduilin was doubly unsaturated as suggested by the infrared spectrum (bands at 1655 and 1582 $cm.^{-1}$) and confirmed by quantitative hydrogenation to tetrahydrobalduilin. The double bond responsible for the relatively strong absorption at 1655 $cm.^{-1}$ is present as an exocyclic methylene group conjugated with a γ-lactone function (infrared band at 1755 $cm.^{-1}$) as in helenalin (I, R = H). The evidence for this is: Ozonolysis of balduilin which has a band in the near infrared

at 1.64 μ[6] gave rise to formaldehyde, whereas tetrahydrobalduilin had no such band and was not attacked by ozone. Comparison of the C-methyl values of balduilin and tetrahydrobalduilin indicated the presence of an additional C-methyl group in the latter. Lastly, the ultraviolet spectrum (ether solution), λ_{max} 218 mμ, ϵ 18,650, was very similar to that of helenalin and had the high-intensity low wave length absorption characteristic of α,β-unsaturated lactones. In view of other results to be discussed in the sequel the maximum at 218 mμ is a composite of two separate chromophores, the α,β-unsaturated ketone chromophore also found in dihydrohelenalin[4] and the conjugated lactone.[7]

Two other oxygen atoms are present as an acetoxy group. In acetonitrile the infrared band at 1755 cm.$^{-1}$ was resolved into a lactone band at 1757 and an acetoxy band at 1747 cm.$^{-1}$; the typical acetate frequency at 1240 cm.$^{-1}$ was also observed. The liberation of acetic acid on hydrolysis of several derivatives of balduilin provides chemical evidence. The hydroxyl group of balduilin involved in ester formation with acetic acid is secondary, as demonstrated by the facile oxidation of the hydrolysis products to ketones with the chromic acid–acetic acid reagent.

The fifth oxygen atom of balduilin is part of a cyclopentenone chromophore. A strong carbonyl band at 1715 (chloroform) or 1712 cm.$^{-1}$ (acetonitrile) disappears on hydrogenation. That the ketone group is still present is shown by the chemical behavior of tetrahydrobalduilin (thioketal formation) and the ultraviolet spectrum (95% ethanol, λ_{max} 283 mμ, ϵ 95). In the infrared, tetrahydrobalduilin had one very strong band at 1750 cm.$^{-1}$ (composite of lactone, acetate and cyclopentanone) which was resolved by acetonitrile into two bands, at 1760 (γ-lactone) and 1740 cm.$^{-1}$ (strength indicative of combined acetate and cyclopentanone). As in the case of helenalin, bromine added easily to the cyclopentenone double bond but not to the α,β-unsaturated lactone (infrared bands at 1755 and 1660 cm.$^{-1}$). Elimination of hydrogen bromide was accomplished readily by refluxing the dibromide in ethanol and the resulting α-bromo-α,β-unsaturated cyclopentenone exhibited maxima at 210 (unsaturated lactone), 251 (α-bromo-α,β-unsaturated ketone) and 315 mμ, ϵ_{max} 11,200, 5530 and 1590.

These observations, and the previously found phytochemical similarity between *Balduina* and *Helenium* species, suggested that balduilin was a guaianolide closely related to helenalin. The difference between the physical properties of balduilin and acetylhelenalin[4] (I, R = Ac) required that the two compounds differ in configuration at one or more of the several centers of asymmetry, in the direction of lactone ring closure (as in II) or both. Moreover tetrahydrobalduilin was not only different from acetyltetrahydrohelenalin (III, R = Ac), but also from dihydroisotenulin (III, R = Ac) or its allo isomer.[8] The interconversions of balduilin, helenalin and tenulin to be discussed now not only proved the original supposition to be correct, but establish that balduilin, helenalin and tenulin possess identical stereochemistry at C_4, C_5, C_7 and C_{10}.

When tetrahydrobalduilin was saponified with potassium hydroxide solution, two desacetyl derivatives were formed which were separated only after extensive chromatography. The preponderant product was desacetyltetrahydrobalduilin A (IV), m.p. 210°, $[\alpha]^{25}D$ 127.2; an isomer, desacetyltetrahydrobalduilin B (V), m.p. 168.5–170°, $[\alpha]^{25}D$ 89°, was isolated in small quantity only.

Chromic acid oxidation of desacetyltetrahydrobalduilin A furnished a diketone (VII) which proved to be identical with a diketone also obtained from tetrahydrohelenalin[4] (III, R = H). Tetrahydrohelenalin on treatment with sodium carbonate gave a new isomer, allotetrahydrohelenalin (VI), m.p. 165.5°, $[\alpha]^{22.5}D$ 125°, infrared bands at 3415 (bonded-OH), 1760 (γ-lactone), 1730 and 1720 cm.$^{-1}$ (cyclopentanone, split band, possibly due to hydrogen bonding.) Oxidation of the latter furnished dehydroallotetrahydrohelenalin (VII), infrared bands at 1760 (γ-lactone), 1750 (cyclopentanone) and 1695 cm.$^{-1}$ (cycloheptanone).

Allotetrahydrohelenalin was subjected to transformations previously[3] carried out with tetrahydrohelenalin, which led to assignment of structure VI. Treatment with ethanedithiol gave an oily thioketal (VIII) which on chromatography over alumina epimerized, presumably at C_{11}, to an isomer IX. Desulfurization of VIII gave an oily desoxoallotetrahydrohelenalin $(X)_1$, $[\alpha]^{22}D$ −20°, whereas desulfurization of IX gave crystalline material (XI), m.p. 134–135°, $[\alpha]^{24}D$ 15°. Chromic acid oxidation of X and XI resulted in liquid ketolactones XII each of which gave an intense Zimmermann test. This indicates that the conversion of tetrahydrohelenalin to allotetrahydrohelenalin involves a change in the closure of the lactone group from C_8 to C_6, the relative configuration of the C_{11}-methyl group in the two compounds being still uncertain.

Desacetyltetrahydrobalduilin A (IV), being convertible to VII, therefore has the same gross structure as allotetrahydrohelenalin (VI) and differs from it only in the configuration of the C_8-hydroxyl group. Since C_8 is not affected by the conversions leading from balduilin to IV and from helenalin to VI, balduilin and helenalin likewise differ at C_8 but have the same stereochemistry at C_4, C_5, C_6, C_7 and C_{10}.

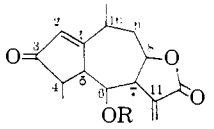
Since acetylation of desacetyltetrahydrobalduilin A and B furnished substances which dif-

(6) W. H. Washburn and M. S. Mahoney, THIS JOURNAL, **80**, 504 (1958).

(7) The ultraviolet spectrum in 95% ethanol had λ_{max} 213 mμ (ϵ 11,900) due to the α,β-unsaturated lactone and λ_{max} 280–285 mμ (ϵ 128), characteristic of a normal ketone. This originally caused us some concern as it appeared to conflict with the other evidence (*vide infra*), but is evidently caused by an unusually facile interaction of the cyclopentenone function of balduilin with hydroxylic solvents. Thus when pure balduilin, m.p. 231°, is dissolved in ethanol and the solvent allowed to evaporate at room temperature, the resulting crystals melt over a wide range, starting near 200°, and gas is evolved. Analysis of this material supports the hypothesis that solvent has been retained. Pure balduilin can again be recovered by recrystallizing from non-hydroxylic solvents. That the cyclopentenone double bond of balduilin is much more reactive than that of helenalin is also shown by the results of catalytic hydrogenation which it was not possible to stop at the dihydro stage. The spectroscopic behavior of brevilin C[1] is similar and may be due to the same cause.

(8) B. H. Braun, W. Herz and K. Rabindran, THIS JOURNAL, **78**, 4423 (1956).

fered from tetrahydrobalduilin it was clear that hydrolysis of tetrahydrobalduilin was accompanied by epimerization of C_{11}, reorientation of the lactone ring or both[9] and that no decision could be made between structures I and II for balduilin on the basis of the now-known structure of desacetyltetrahydrobalduilin A. The difficulty was compounded by the following series of experiments.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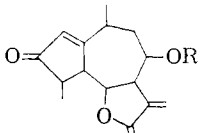
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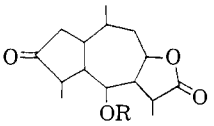
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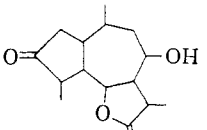
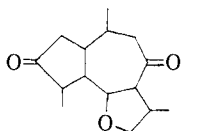
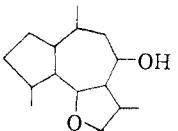
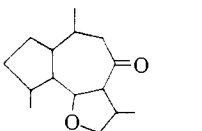
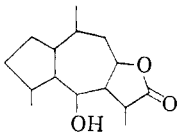
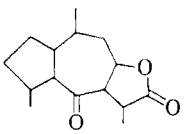

IV, VI


VII


X, XI


XII


XIV


XV

Desulfurization of the crystalline thioketal of tetrahydrobalduilin furnished desoxotetrahydrobalduilin (XIII), infrared bands at 1760 (γ-lactone) and 1737 cm.$^{-1}$ (acetate). Hydrolysis of XIII also required aqueous sodium hydroxide. The product proved to be 6-epidesoxodesacetyldihydroalloisotenulin[10] of known structure (XIV) and confirmation was provided by oxidation to dehydrodesoxodesacetyldihydroalloisotenulin[8] (XV). Thus the compounds of the balduilin series are C_6-epimers of the tenulin series, the configuration at C_4, C_5, C_7, C_8 and C_{10} being the same. But since XIII was different from the acetate of XIV, hydrolysis was again accompanied by epimerization at C_{11}, lactone reorientation or both and the question of the orientation of the lactone ring of balduilin remained unsettled.

It is hoped to investigate this point more fully when additional material becomes available. In the meantime, the isolation of balduilin provides the "missing link" which has served to define clearly the stereochemical differences between helenalin and tenulin. The conversion of balduilin to dehydroallotetrahydrohelenalin (VII) on the one hand and to dehydrodesoxodesacetyldihydroalloisotenulin (XV) on the other demonstrates that the two guaianolides from *Helenium* species have the same configuration at C_4, C_5, C_7 and C_{10} and the opposite configuration at C_6 and C_8. The absolute configuration of tetrahydrobalduilin at C_1, C_5 and C_7 is the same as that previously derived for tetrahydrohelenalin and dihydroisotenulin.[11]

(9) In the tenulin series methanolic potassium carbonate suffices for the removal of the acetate group,[10] conditions which are not sufficiently drastic to cause epimerization or relactonization. The hydrolysis of balduilin and its derivatives requires more rigorous conditions. Everything else being equal the configuration of C_6, whether involved in lactone or ester formation, seems responsible.

(10) W. Herz, K. Rabindran and W. A. Rohde, to be published.

ADDED IN PROOF.—A. Romo de Vivar and J. Romo, *Chem. Ind.*, 882 (1959), recently reported the isolation, from *Helenium mexicanum* H.B.K., of helenalin and four new substances, mexicanin A, B, C and D. The physical properties of dihydromexicanin C and dihydromexicanin L resembled those of VI and VII. A direct comparison of dehydrodihydromexicanin C and VII, kindly carried out by Dr. Romo, has demonstrated their identity, from which it follows that the structure of mexicanin C is dihydro-II (R = H).

Acknowledgments.—This work was supported in part by grants from the National Science Foundation (G-3313) and the United States Public Health Service (RG-5814) for which we express our thanks. We are greatly indebted to Dr. Robert K. Godfrey for help with the collection and identification of *B. uniflora* Nutt. and for drawing our attention to the taxononic problem.

Experimental[12]

Balduilin.—*Balduilin uniflora* Nutt. was collected near St. Marks, Florida, in September, 1957 and 1958. The dried flowers, leaves and stems, wt. 2.5 lbs., were extracted with 5 l. of chloroform in a large Soxhlet extractor for two days. The extract was freed from chloroform. The residue was treated with a mixture of 300 ml. of hot 95% ethanol, 500 ml. of hot water and 7 g. of lead acetate dissolved in a small amount of water acidified with 2 ml. of acetic acid. After allowing to settle overnight the pale yellowish-brown supernatant solution was filtered. The filtrate was concentrated under reduced pressure, extracted thoroughly with chloroform and the chloroform extract washed and dried. Removal of chloroform resulted in 5.5 g. of a crude semi-solid product which was chromatographed over 30 g. of basic alumina (solvent and eluent benzene). From the first five fractions of 50 ml. each was obtained a total of 1.43 g. of crystalline material, the m.p. of samples from the various fractions varying from 220 to 230°. Recrystallization from benzene gave colorless rhombs, m.p. 231–232°, $[\alpha]^{24}D$ 57° (chloroform, *c* 1.05); infrared bands at 1755 double strength, (γ-lactone and acetoxy), 1715 (cyclopentenone), 1655 (C=C), 1582 (C=C) and 1408 cm.$^{-1}$; (in acetonitrile) 1757, 1747, 1712, 1240 cm.$^{-1}$. The ultraviolet spectrum was discussed previously.

Anal. Calcd. for $C_{17}H_{20}O_5$: C, 67.09; H, 6.62. Found: C, 67.09; H, 6.87; C-methyl, 2.42 moles.

From a total of 10 lb. 12 oz. of dried plant there was isolated 7.03 g. (0.15%) of balduilin. Balduilin gives a slow positive Zimmermann test. On ozonolysis in the usual way, 122 mg. of balduilin gave 30 mg. (26%) of the dimedone derivative of formaldehyde.

Dibromobalduilin.—A solution of 0.200 g. of balduilin in 10 ml. of chloroform was treated with 5 ml. of chloroform containing 0.145 g. of bromine until the color of bromine persisted. The solvent was removed *in vacuo* and the residual

(11) C. Djerassi, J. Osiecki and W. Herz, *J. Org. Chem.*, **22**, 1361 (1957).

(12) M.p.'s and b.p.'s are uncorrected. Analyses by Drs. Weiler and Strauss, Oxford, Eng. Infrared spectra were run in chloroform solution and ultraviolet spectra in 95% ethanol unless otherwise specified.

oil triturated with petroleum ether (b.p. 30–60°). Recrystallization from benzene–petroleum ether furnished a colorless product, m.p. 167°.

Anal. Calcd. for $C_{17}H_{20}Br_2O_5$: C, 43.98; H, 4.34; Br, 34.43. Found: C, 44.08; H, 4.23; Br, 33.45.

Bromobalduilin.—A mixture of 0.10 g. of dibromobalduilin and 10 ml. of 50% ethanol was refluxed for one hour. The solution became acid. On cooling there precipitated a solid which was recrystallized from benzene–petroleum ether and then melted at 224° dec.

Anal. Calcd. for $C_{17}H_{19}BrO_5$: C, 53.27; H, 4.99; Br, 20.8. Found: C, 53.07; H, 5.00; Br, 21.2.

Tetrahydrobalduilin.—A solution of 0.735 g. of balduilin in 50 ml. of ethyl acetate was hydrogenated with 50 mg. of prereduced platinum oxide catalyst at atmospheric pressure; calculated hydrogen uptake (23.5°) for two double bonds 118 ml., observed 127 ml. The solvent was removed and the residue, m.p. 162–164°, recrystallized repeatedly from cyclohexane–acetone. The analytical sample melted at 169–170°, $[\alpha]^{24}D$ 52.5°; infrared bands at 1750 (broad and unsymmetrical) and 1408 cm.$^{-1}$ (in acetonitrile) 1762, 1747 (shoulder at 1750) and 1245 cm.$^{-1}$. The m.p. of tetrahydrobalduilin is somewhat variable, occasional later preparations resulting in samples melting as high as 177–178°. The infrared spectra of these fractions were indistinguishable from the spectrum of the material of m.p. 169–170°.

Anal. Calcd. for $C_{17}H_{24}O_5$: C, 66.21; H, 7.85. Found: C, 65.88; H, 7.93; C-methyl, 3.39 moles.

The substance gave a strong positive Zimmermann test, but failed to give a derivative on attempted condensation with piperonal. Attempts to deacetylate it with potassium carbonate in methanol at room temperature for 24 hours and at reflux temperature for 1 hour, or with aqueous acid, were unsuccessful and only starting material was recovered.

Hexahydrobalduilin.—A suspension of 2.88 g. of balduilin in 100 ml. of ethyl acetate was reduced in the Parr hydrogenerator at 50–60 lb. pressure with platinum oxide catalyst. The material dissolved on hydrogenation. The solution was concentrated to 10 ml. and the crystalline product, m.p. 197–200°, filtered, wt. 2.55 g. Several recrystallizations from acetone–cyclohexane raised the m.p. to 202–203°, $[\alpha]^{23}D$ −62.4° (95% ethanol, *c* 0.50); infrared bands at 3450 (hydroxyl), 1760 (lactone) and 1725 cm.$^{-1}$ (acetoxy). Chromic acid oxidation furnished tetrahydrobalduilin.

Anal. Calcd. for $C_{17}H_{26}O_5$: C, 65.78; H, 8.44. Found: C, 65.90; H, 8.41.

Desacetyltetrahydrobalduilin A and B.—A mixture of 1.0 g. of tetrahydrobalduilin and 16 ml. of 1 *N* sodium hydroxide solution was refluxed for 15 minutes (development of a red color), cooled, diluted with 5 ml. of water and neutralized carefully with dilute hydrochloric acid. The mixture was concentrated *in vacuo*, cooled and extracted with benzene. The benzene layer was dried; the solvent was removed and the residual semi-solid material, wt. 0.75 g., chromatographed over 10 g. of basic alumina. The first 25 ml. of eluate gave 200 mg. of crystalline desacetyltetrahydrobalduilin A, m.p. 203–208°. From the subsequent fractions was collected 130 mg. of crude desacetyltetrabalduilin B, m.p. 155–162°, and gum which would not crystallize. Recrystallization of A from benzene–ligroin (b.p. 60–90°) gave colorless prisms, m.p. 203–205°, $[\alpha]^{25}D$ 127° (95% ethanol, *c* 0.50); infrared bands at 3440 (hydroxyl), 1762 (lactone), 1735 (cyclopentanone, shoulder at 1725-bonded cyclopentanone?) and 1412 cm.$^{-1}$.

Anal. Calcd. for $C_{15}H_{22}O_4$: C, 67.44; H, 8.41. Found: C, 67.64; H, 8.33.

Isomer B was recrystallized from benzene–ligroin. The product melted at 168–170° (depression to 122–127° on admixture of allotetrahydrohelenalin), $[\alpha]^{25}D$ 89° (95% ethanol, *c* 0.33); infrared bands at 3450, 1760, 1740 (shoulder at 1727) and 1410 cm.$^{-1}$.

Anal. Calcd. for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.76; H, 8.28.

Subsequent hydrolyses of tetrahydrobalduilin required extensive rechromatography over acid-washed alumina before desacetyltetrahydrobalduilin B was obtained in pure form. In a typical run, 750 mg. of tetrahydrobalduilin gave 220 mg. of isomer A, m.p. in the range 200–210°, eluted with benzene. Later fractions were eluted with benzene–ether varying in concentration from 97:3 to 9:1. The earlier portions yielded material melting in the range 190–200° which was rechromatographed and recrystallized to give an additional 0.025 g. of isomer A. Material from the later fractions melted in the range 140–165°. On recrystallization this furnished 0.06 g. of isomer B.

Isomer A could not be acetylated under the mild conditions successful with tetrahydrohelenalin and desacetyldihydroisotenulin. A mixture of 0.16 g. of isomer A, 0.06 g. of fused sodium acetate and 10 ml. of acetic anhydride was refluxed for three hours, cooled, and excess anhydride removed *in vacuo*. The residue was dissolved in benzene and chromatographed over basic alumina, developed with benzene and eluted with benzene–ether (95:5). Recrystallization of the product furnished white needles, m.p. 170–171° depressed to 145–150° on admixture of tetrahydrobalduilin), $[\alpha]D$ 72° (95% ethanol, *c* 0.20); infrared bands at 1775, 1750 and 1740 cm.$^{-1}$.

Anal. Calcd. for $C_{17}H_{24}O_5$: C, 66.21; H, 7.84. Found: C, 66.37; H, 7.21.

Acetylation of isomer B gave an oil which could not be induced to crystallize, infrared bands at 1765 (γ-lactone) and 1735 cm.$^{-1}$ (double strength, cyclopentanone and acetoxy), $[\alpha]^{25}D$ −17° (95% ethanol, *c* 0.60).

Anal. Calcd. for $C_{17}H_{24}O_5$: C, 66.21; H, 7.84. Found: C, 66.47; H, 7.96.

Oxidation of isomer A with chromic acid in acetic acid gave dehydroallotetrahydrohelenalin, m.p. 166°, $[\alpha]^{25}D$ 27.3 (95% ethanol, *c* 0.365); infrared bands (in acetonitrile) at 1762 (γ-lactone), 1747 (cyclopentanone) and 1695 (rel. weak, cycloheptanone).

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 67.84; H, 7.54.

Desoxotetrahydrobalduilin.—Tetrahydrobalduilin, wt. 0.50 g., was mixed with 0.75 ml. of ethanedithiol and 1.5 ml. of boron trifluoride-etherate. After one hour at room temperature the mixture was diluted with water and extracted with ether. The ether extract was washed with water, 3% sodium hydroxide solution, again with water, dried and concentrated to 3–4 ml., when the thioketal crystallized. The product was washed with 3 ml. of cold ether; yield 0.52 g. of rhomboids, m.p. 178–182°. Two recrystallizations from aqueous methanol gave the analytical sample, m.p. 183–184°, infrared bands (in acetonitrile) at 1760 and 1740 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{28}O_4S_2$: C, 59.34; H, 7.34. Found: C, 59.42; H, 7.50.

A solution of 0.20 g. of the thioketal in 30 ml. of absolute ethanol was refluxed with Raney nickel for 13 hours. The catalyst was removed by filtration through a bed of infusorial earth and the filtrate and wastings were evaporated. The residue crystallized on standing, m.p. 165–166°, yield 0.15 g. Two recrystallizations from ligroin (b.p. 60–90°) furnished the analytical sample, $[\alpha]D$ 72° (95% ethanol, *c* 0.365), infrared bands at 1760 and 1737 cm.$^{-1}$.

Anal. Calcd. for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90. Found: C, 69.19; H, 8.75.

Desoxodesacetyltetrahydrobalduilin (6-Epidesoxodesacetyldihydroalloisotenulin).—A solution of 220 mg. of desoxotetrahydrobalduilin in 3 ml. of ethanol and 15 ml. of 4% sodium hydroxide solution was refluxed for 25 minutes. Ethanol was removed and the mixture cooled, acidified with dilute hydrochloric acid and then made basic with sodium bicarbonate to remove the acetic acid which had been generated. The product was extracted with ether, washed and dried. Removal of the ether yielded an oil which was chromatographed over 4 g. of basic alumina, solvent and eluent benzene–ligroin (7:3). The first four fractions of eluate (150 ml.) yielded 37 mg. of crystalline material, m.p. 157°, mixed m.p. with authentic 6-epidesoxodesacetyldihydroalloisotenulin[10] undepressed. The infrared spectra were superimposable in every detail.

Chromic acid oxidation of 50 mg. of the hydrolysis product gave 35 mg. of dehydrodesoxodesacetyldihydroalloisotenulin, m.p. 129–130°, undepressed mixed m.p. with an authentic sample,[8] m.p. 130–131°. The infrared spectra were superimposable.

Later fractions of the chromatogram yielded crystalline material which melted unsharply below 140° and could not be recrystallized to constant m.p., apparently due to contamination by another isomer. That the hydrolysis

conditions are sufficient to cause at least epimerization at C_{11} was shown by the following experiment. A mixture of 100 mg. of 6-epidesoxodesacetyldihydroisotenulin,[10] 3 ml. of ethanol and 0.1 ml. of 10% sodium hydroxide solution was heated on the steam-bath for 30 minutes. Removal of ethanol followed by cooling and careful dilution with water caused gradual crystallization of 6-epidesoxodesacetyldihydroalloisotenulin, m.p. 157–158°.

Allotetrahydrohelenalin.—A mixture of 0.15 g. of tetrahydrohelenalin,[4] 0.25 g. of sodium carbonate, 3 ml. of water and 0.5 ml. of ethanol was refluxed for 15 minutes. The solid tetrahydrohelenalin dissolved within 5–10 minutes. The alcohol was driven off and the solution was cooled and acidified with concd. hydrochloric acid. The semi-solid product was taken up in chloroform, the chloroform layer was washed, dried and concentrated *in vacuo*. The residual oil was crystallized from aqueous methanol to give 0.07 g. of colorless thick prisms, m.p. 161–163°, mixed m.p. with tetrahydrohelenalin depressed to 140°. Three additional crystallizations from aqueous methanol gave needles, m.p. 165.5°, $[\alpha]^{23}D$ 125° (95% ethanol, *c* 0.56); infrared bands at 3415 (bonded OH), 1760 (γ-lactone), 1730 (cyclopentanone, shoulder at 1720-bonded?) and 1412 cm.$^{-1}$.

Anal. Calcd. for $C_{15}H_{22}O_4$: C, 68.49; H, 8.03. Found: C, 68.64; H, 8.33.

Dehydroallotetrahydrohelenalin.—Chromic acid oxidation of 100 mg. of allotetrahydrohelenalin yielded 80 mg. of dehydroallotetrahydrohelenalin, m.p. 170°, $[\alpha]^{23}D$ 33.3° (95% ethanol, *c* 0.605), mixed m.p. with material from the oxidation of desacetyltetrahydrobalduilin A (m.p. 166°) 167–168°. The infrared spectra of the two samples were superimposable in every detail.

Desoxoallotetrahydrohelenalin.—A mixture of 0.1 g. of allotetrahydrohelenalin, 0.15 ml. of ethanedithiol and 0.25 ml. of boron trifluoride-etherate was allowed to stand for one hour, diluted with water and extracted with ether. The ether extract was washed with water, aqueous sodium hydroxide and again with water. Removal of solvent yielded an oil which refused to crystallize; wt. 0.11 g., infrared bands at 3450 and 1760 cm.$^{-1}$. The oil, wt. 0.31 g., was desulfurized with Raney nickel and worked up in the usual way to give 0.25 g. of an oil which refused to crystallize. It was purified by chromatography over alumina (solvent and eluent benzene), $[\alpha]^{22}D$ −20° (95% ethanol, *c* 0.53), infrared bands at 3450 and 1760 cm.$^{-1}$.

Anal. Calcd. for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59. Found: C, 71.18; H, 9.47.

When the thioketal was chromatographed over alumina, there were some changes in the fingerprint region of the infrared spectrum. Desulfurization of 100 mg. of the thioketal, after chromatography and purification in the usual manner, yielded 80 mg. of a crystalline residue which was recrystallized from benzene–ligroin and ligroin. The felted needles melted at 134–135°, $[\alpha]^{24}D$ 15° (95% ethanol, *c* 0.28); infrared bands at 3570 and 3450 (non-bonded and bonded OH) and 1750 cm.$^{-1}$ (γ-lactone). There were significant differences in the fingerprint region of the two hydroxylactones.

Anal. Calcd. for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59. Found: C, 71.96; H, 9.12.

Dehydrodesoxoallotetrahydrohelenalin.—Oxidation of 0.19 g. of the non-crystalline desoxoallotetrahydrohelenalin in 10 ml. of acetic acid with 5.2 ml. of a 1.33% solution of chromic acid in acetic acid was allowed to proceed at 5° overnight. Excess oxidizing agent was destroyed by adding methanol and the combined solvents were removed. The residue was diluted with water and extracted with ether. The ether extract was washed, dried and freed of solvents to give 0.155 g. of a viscous oil (isomer A) which refused to crystallize; $[\alpha]^{23}D$ −13.2° (95% ethanol, *c* 0.295), infrared bands at 1765 (γ-lactone) and 1700 cm.$^{-1}$ (cycloheptanone). The material gave a positive Zimmermann test.

Anal. Calcd. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 72.42; H, 8.91.

Oxidation of 0.35 g. of the solid hydroxylactone in the same manner gave 0.33 g. of viscous ketolactone, $[\alpha]^{23}D$ 43.2° (95% ethanol, *c* 0.665). It gave a positive Zimmermann test. The infrared spectrum of this material (isomer B) did not differ significantly from that of the isomer A, the fingerprint region being rich in detail, although the rotation was quite different. In an attempt to prepare the oil for analysis, it was distilled in a high vacuum. Two fractions were collected: fraction 1, b.p. 135–143° (bath temp., 0.03 mm.), $[\alpha]^{24}D$ 42° (95% ethanol, *c* 0.3); and fraction 2, b.p. 155–160° (bath temp., 0.02 mm.), $[\alpha]^{24}D$ 75.8° (95% ethanol, *c* 0.545). It is not clear whether isomerization occurred during the distillation or whether the original material was a mixture. The infrared spectra of the two fractions were practically indistinguishable from each other and from the spectrum of isomer A.

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A Chromatographic Study of the Minor Components of Normal Adult Human Hemoglobin Including a Comparison of Hemoglobin from Normal and Phenylketonuric Individuals

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An improved procedure is described for the separation of minor hemoglobin components in hemolysates of red blood cells and hence for the purification of the main component. A comparison of the minor components of hemoglobin from normal and phenylketonuric individuals has failed to reveal any significant differences in the number or amounts. Thus, the abnormal amounts of free phenylalanine in the organism have failed to influence the biosynthesis of the hemoglobin significantly.

Introduction

It is now well established that the hemoglobin from a hemolysate of normal adult human red blood cells is heterogeneous. This fact, although suspected earlier on the basis of experiments that did not permit the isolation of the components,[1–3] received its first unequivocal demonstration when Kunkel and Wallenius[4] separated minor fractions from human adult hemoglobin by starch block electrophoresis. At about the same time, by means of chromatography on the ion-exchange resin, IRC-50, Morrison and Cook[5] found a component (10%) that moved more rapidly and another (6%) that moved more slowly than the main component. Huisman and Prins[6] detected only the faster component with

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