tion was filtered while hot to remove a small amount of solid, probably disulfide, which did not dissolve. The solid which separated from the filtrate on cooling was collected by filtration and dried in a vacuum desiccator to give 18.0 g. (25% of theoretical) of 5-mercaptouracil.

Anal. Calcd. for $C_4H_4N_2O_2S$: C, 33.3; H, 2.80; N, 19.4; S, 22.2. Found: C, 33.2; H, 2.56; N, 19.1; S, 22.5.

b. 5-Uracilyl Disulfide.—Fifty-six grams (0.5 mole) of uracil was chlorosulfonated and reduced as described above with the exception that only 125 g. of zinc was used and the reduction mixture was refluxed for 5 hours. The crude product was collected by filtration, precipitated from 5% sodium carbonate solution with acetic acid and recrystallized from water using a Soxhlet extractor to give 29.6 g. (41% of theoretical) of 5-uracilyl disulfide.

Anal. Calcd. for $C_8H_6N_4O_4S_2$: C, 33.6; H, 2.12; N, 19.6; S, 22.5. Found: C, 33.2; H, 2.22; N, 19.3; S, 22.7.

CHICAGO 9, ILLINOIS

[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Jervine. IX. Miscellaneous New Derivatives

BY B. M. ISELIN,[1] M. MOORE AND O. WINTERSTEINER

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The steric course of the reduction of the 11-keto group in tetrahydrojervine (I) parallels that in normal 11-keto-steroids in that reduction with sodium and butanol leads to the 3,11α-diol II (the "β-tetrahydrojervinol" of Jacobs and Huebner),[2] in which the 11-hydroxyl group is unhindered, while with lithium aluminum hydride the hindered 11β-epimer IV is formed. Jervine itself is reduced by the latter reagent to a mixture of dihydrojervine and the unsaturated diol V in which the 11-hydroxyl group appears to be likewise β-oriented. Two, probably stereoisomeric, forms of diacetyljervine 5,6-dibromide and the 3-monoacetates of jervine, dihydrojervine and tetrahydrojervine are described.

The purpose of this paper is to place on record several new derivatives of jervine which were prepared in an early phase of our investigations (1950) but played no direct role in the elucidation of its structure. They all can be formulated readily in terms of the now accepted structure for jervine.

One of our objects at that time was to clarify the relationship between two supposedly 11-epimeric, singly unsaturated diols which Jacobs and Huebner[2] had obtained by reduction with sodium and butanol of jervine and (13,17a)-dihydrojervine, respectively. The diol obtained in this manner from jervine itself ("α-dihydrojervinol") was believed to have suffered reduction at the 11-keto group as well as at the double bond conjugated with it, that from dihydrojervine ("β-dihydrojervinol") at the keto group only. "α-Dihydrojervinol," save for the preparation of a N-acetate, was not further investigated. "β-Dihydrojervinol" formed a triacetate, showing that the new hydroxyl derived from the inert keto group was not hindered. On catalytic reduction it gave a saturated diol ("β-tetrahydrojervinol"), which could also be obtained by sodium–butanol reduction of tetrahydrojervine, and on Oppenauer oxidation an α,β-unsaturated monoketone ("Δ^4-β-dihydrojervinol").[3]

When "α-dihydrojervinol" was prepared in our laboratory, it soon became apparent that the properties and reactions of this compound were incompatible with its formulation as a singly (Δ^5)-unsaturated 3,11-diol, and that it was in fact a dienic triol in which the oxidic ring of jervine has been opened by hydrogenolysis.[4] A reinvestigation also of the "β"-series seemed then in order, the more so as it had meanwhile been shown[5] that jervine could not be a normal 11-keto-steroid. To avoid complications in the projected reoxidation to ketonic derivatives, the saturated ketone, tetrahydrojervine (I), rather than dihydrojervine, was used as the starting material. On reduction with sodium and butanol it readily afforded "β-tetrahydrojervinol" (II) as described by Jacobs and Huebner. This diol formed on acetylation with acetic anhydride and pyridine a triacetate (IIa, m.p. 175–178°, $[\alpha]^{23}D$ + 69°), behaving in this respect like "β-dihydrojervinol." The N-acetate (IIb, m.p. 257–259°, $[\alpha]^{25}D$ +84°), prepared by selective N-acetylation, was oxidized with chromic acid to the 3,11-diketone, N-acetyltetrahydrojervone (III, m.p. 267–271°, $[\alpha]^{25}D$ +27.5°), identical with the product obtained in the same manner from N-acetyltetrahydrojervine. The two specimens yielded the same mono-2,4-dinitrophenylhydrazone, m.p. 255–256°. It was clear, then, that the oxidic linkage in tetrahydrojervine, unlike that in jervine, had not been affected by the reduction with sodium, and that "β-tetrahydrojervinol" was the normal saturated diol with an unhindered 11α-hydroxyl group.

On reduction with lithium aluminum hydride tetrahydrojervine as expected yielded the 11-epimer of II, the 3β,11β-diol IV (m.p. 246.5–248.5°, $[\alpha]^{21}D$ +25°). Careful chromatographic fractionation showed that the epimer II was not present in appreciable amounts.[5a] The acetylation product of IV was amorphous, but its acetyl content and the fact that on chromic acid oxidation it gave diacetyltetrahydrojervine (Ia) left no doubt that it was the diacetate IVa in which the 11-hydroxyl group had remained unacetylated.

It is clear, therefore, that the reduction of the 11-keto group in tetrahydrojervine takes the same steric course as with normal 11-keto-steroids, in that reduction with sodium (or lithium) establishes the thermodynamically more stable unhindered α-

(1) Ciba Research Laboratories, Basel, Switzerland.

(2) W. A. Jacobs and C. F. Huebner, *J. Biol. Chem.*, **170**, 635 (1947).

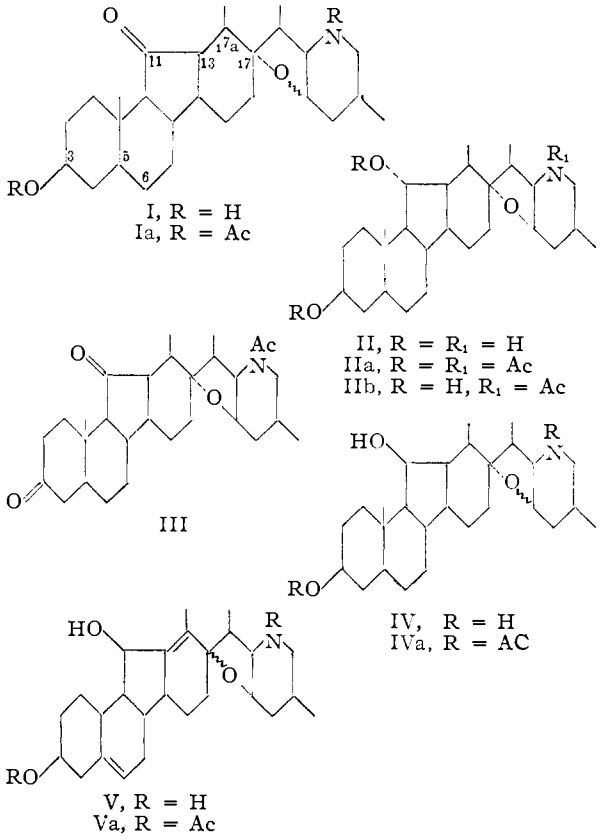
(3) W. A. Jacobs and Y. Sato, *ibid.*, **175**, 57 (1948).

(4) J. Fried and A. Klingsberg, to be published.

(5) O. Wintersteiner, M. Moore, J. Fried and B. M. Iselin, *Proc. Nat. Acad. Sciences*, **37**, 333 (1951).

(5a) This is in contrast to the experience in the lithium aluminum hydride reduction of cortisone; *cf.* R. Antonucci, S. Bernstein, M. Lenhard, R. Littell and J. H. Williams, *J. Org. Chem.*, **18**, 70 (1953).

configuration with equatorial (in II quasi-equatorial) conformation of the hydroxyl group, while that with metal hydrides gives rise to the β-configuration in which the hydroxyl group is axial (in IV quasi-axial) and strongly hindered by the 19-methyl group. The hindrance relations are fully borne out by the scale models of II and IV, con-



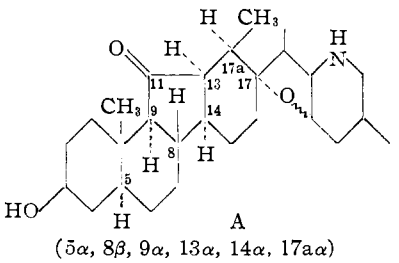
structed on the premise that rings B and C are *trans*-linked as in normal steroids (8β, 9α).[6] Table I lists the molecular rotation data for the 11-epimeric tetrahydrojervinols[7] and tetrahydrojervine together with those for representative 11-epimeric alcohols and 11-ketones of the normal series. It will be noted that while in the normal series the 11α-epimers are more levorotatory than the β-epimers, the reverse is true of the 11-epimeric tetrahydrojervinols. Likewise, in the normal series the 11-ketones are more dextrorotatory, and tetrahydrojervine is more levorotatory, than either of the corresponding 11-epimeric alcohols. The fact that in the tetrahydrojervine derivatives C-11 is

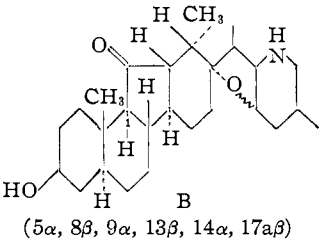
TABLE I

Compound	$[M]_D^{Chlf.}$	(11α-OH)-(11β-OH)	$\Delta[M]_D$ (11α-OH)-(11-ketone)	(11β-OH)-(11-ketone)
Tetrahydrojervin-11α-ol	+170°			
Tetrahydrojervin-11β-ol	+108°	+ 62°	+308°	+246°
Tetrahydrojervine	−138°			
11α-Hydroxyprogesterone[a]	+588°			
11β-Hydroxyprogesterone[a]	+714°	−126°	−318°	−192°
11-Ketoprogesterone[b]	+906°			
22a-5α-Spiran-3β,11α-diol[c]	−298°			
22a-5α-Spiran-3β,11β-diol[d]	−212°	− 86°	−169°	− 83°
22a-5α-Spiran-3β-ol-11-one[c]	−129°			

[a] J. Fried, R. W. Thoma, J. R. Gerke, J. E. Herz, M. N. Donin and D. Perlman, THIS JOURNAL, 74, 3962 (1953). [b] Own measurement. [c] F. Sondheimer, O. Mancera, G. Rosenkranz and C. Djerassi, THIS JOURNAL, 75, 1282 (1953). [d] C. Djerassi, E. Batres, M. Velasco and G. Rosenkranz, *ibid.*, 74, 1712 (1952).

(6) We have come to favor either A or B below as the most probable steric expressions for tetrahydrojervine (I), on the basis of the following considerations: (1) There can be little doubt from the mode of preparation of I from dihydrojervine (catalytic reduction of 5,6-double bond with PtO_2 in acetic acid) that rings A and B are *trans*-linked; (2) it stands to reason on biogenetic grounds that the configurations of C-8, C-9, C-14 and C-17 are the same as in normal steroids. Moreover, some evidence that rings B and C are *trans*-linked is at hand (B. M. Iselin and O. Wintersteiner, Paper VIII of this series, THIS JOURNAL, 77, 5318 (1955)); (3) in the absence of the hindrance exerted in normal steroids by the β-oriented angular 18-methyl group, the hydrogen added to the 13,17a-double bond in the formation of dihydrojervine from jervine (PtO_2, ethanol) could enter either from the rear (A) or from the front (B). Of these, A deserves perhaps preference on the grounds that the *cis*-form of α-hydrindanones is generally more stable than the *trans*-form (W. Hückel, M. Sadis, J. Vantschulevitsch and F. Nerdel, *Ann.*, 518, 155 (1935)), and that the bulky C-20, 21-side chain grouping, assumed to be β-oriented, might impede the attachment of the catalyst at the β-face.





From the scale models (Catalin Ltd., London) of I, and of the two 11-epimeric diols II and IV, when first given the configurations of form A, and then those of form B, the following information can be gleaned.

Form A.—Provided that ring D is made a boat, the A, B, C, D ring system is almost strainless, and, except for a slight forward tilt of ring D, fairly planar. The keto group in I is strongly interfered with by the 19-methyl group and the β-hydrogen atom at C-1; the (axial) 11β-hydroxyl in IV by the 19- as well as by the (axial) 18-methyl group. The (equatorial) 11α-hydroxyl in II is completely unhindered.

Form B.—This form (with ring D as chair) is considerably more strained around ring C than A, but not prohibitively so. The ring system is completely planar. The 11-carbonyl and (axial) 11β-hydroxyl abut against the 19-methyl group and the C-1 β-hydrogen, but the former is also shielded to some extent from the rear by the α-oriented (axial) 18-methyl. However, in this form, also the 11α-hydroxyl appears to be encumbered by the latter methyl group, though it is fully accessible from the front of the molecule.

While it would lead too far afield to discuss in detail the four forms corresponding to A and B in which rings B and C are *cis*-linked, it might be mentioned that in two of these (A, 8α, 9α and A, 8β, 9β) the 11β-hydroxyl is strongly, and the 11α-hydroxyl not or only moderately, hindered; in one (B, 8β, 9β) this relationship is reversed; and in the fourth (B, 8α, 9α) there is moderate hindrance of the hydroxyl in either configuration. The above experimental results do therefore not *per se* prove that rings B and C are *trans*-linked.

(7) Since the rational nomenclature devised by us for jervine and its derivatives (J. Fried and A. Klingsberg, THIS JOURNAL, 75, 4929 (1953)) is somewhat cumbersome, we are retaining the trivial names "jervinol," "dihydrojervinol" and "tetrahydrojervinol" for the 3,11-diols derived from jervine and its hydrogenated derivatives, but in conjunction with the prefixes 11α and 11β before "ol" to designate the true configuration of the 11-hydroxyl group.

flanked by two, instead of one, asymmetric centers is probably in part responsible for the change of sign of the molecular rotation differences mentioned.

The reduction of jervine itself with lithium aluminum hydride gave besides oils a crystalline mixture the main component of which was identified as dihydrojervine. The other substance present (m.p. 278–283°, $[\alpha]^{25}D$ −70° in ethanol) likewise lacked jervine absorption. The analysis, the absence of a keto band in the infrared spectrum and the fact that it gradually decomposed on storage left little doubt that it was one of the 11-epimeric allylic alcohols, *i.e.*, a jervinol. The sirupy acetylation product was purified by chromatography, when it yielded a crystalline substance (m.p. 213–217°, $[\alpha]^{25}D$ −36°) which analytically appeared to be a jervinol diacetate. That it deteriorated rapidly on standing may also be taken as an indication that the allylic hydroxyl group was free. The reduction product is therefore in all likelihood jervine-11β-ol (V), and the acetylated substance its 3,N-diacetate (Va).

One of our early objectives was to locate the keto group of jervine by oxidative degradation of the ring carrying that group. Since diacetyltetrahydrojervine proved to be very resistant to oxidation, it became necessary to conduct these experiments on diacetyljervine after protection of the latter's 5,6-double bond by bromination. The diacetate readily added 2 atoms of bromine in acetic acid solution with the formation of two (stereoisomeric?) dibromides separable by virtue of their different solubilities in ether. The component preponderating in the mixture (B, m.p. 146–147°, $[\alpha]^{24}D$ −68°, yield 47% of theory) was readily soluble in ether, while that present in smaller amounts (A, m.p. 157–158°, $[\alpha]^{25}D$ −46°, yield 4.5%) remained in the insoluble fraction. The fact that the ultraviolet absorption spectrum of both products was identical with that of jervine, and that they both could be reconverted in good yield to diacetyljervine by debromination with zinc in ethanol showed conclusively that they were 5,6-dibromides. From their mode of formation it must be presumed that both isomers are *trans*-dibromides; hence they should correspond to the two known 5,6-dibromides derived from cholesterol which Barton and Miller[8] have shown to be, respectively, the (diequatorial, stable) 5β,6α-dibromocoprostanol and the (diaxial, unstable) 5α,-6β-dibromocholestanol. However, there is no good agreement in the molecular rotation changes attending the formation of the isomeric dibromides from their unsaturated parent compounds in the two series. While for the more dextrorotatory dibromides of each pair (diacetyljervine dibromide A, 5β,6α-dibromocoprostanol, 5β,6α-dibromocoprostanyl benzoate) the molecular rotation differences for the bromine addition to the 5,6-double bond agree at least in sign, the respective values being +248°, +408° and +597°, this is not the case for their more levorotatory counterparts (diacetyljervine dibromide B, 5α,6β-dibromocholestanol, 5α,6β-dibromocholestanyl benzoate) where the respective $\Delta[M]D$ values are +100°, −89°, and −151°.[9] This may be simply due to constitutional factors which manifest themselves more strongly in the diaxial dibromide B (6β-bromine-19-methyl interference), or the latter may be actually a mixture containing a good proportion of dibromide A (A and B form mixed crystals as indicated by mother liquor fractions having intermediate melting points and rotations). No tests for stability to alkali, etc.,[8] were carried out, but that B is the diaxial and hence unstable isomer would appear from the fact that on storage for several years it had turned into a black tar, while a sample of A in the same interval had become slightly yellow and after one recrystallization showed the original melting point.

Free jervine on bromination in acetic acid yielded a 5,6-dibromide hydrobromide, m.p. 217–219°, $[\alpha]^{25}D$ −88° in 50% aqueous ethanol. It is noteworthy that in this case λ_{max}^{alc} is hypsochromically displaced to 245 mμ, and the extinction is raised to 18,300 while the unbrominated hydrobromide (m.p. 277–279°) and hydrochloride show the same ultraviolet characteristics as the free base (λ_{max}^{alc} 250 mμ, ϵ 15,000).

In connection with an attempt to reduce the α,β-unsaturated keto system of jervine with zinc and acetic acid it was observed that prolonged refluxing (18 hours) of the base with acetic acid alone afforded in about 45% yield a compound m.p. 277–279°, $[\alpha]^{27}D$ −138° in ethanol which was identified as 3-monoacetyljervine by analysis, the spectral characteristics (ultraviolet spectrum unchanged, appearance of ester bands at 5.79 and 7.98 μ in the infrared spectrum) and its reconversion to jervine by alkaline hydrolysis. The 3-monoacetates of dihydrojervine (m.p. 280–282°, $[\alpha]^{24}D$ −100°) and of tetrahydrojervine (m.p. 250–252°, $[\alpha]^{25}D$ −48°) were prepared in the same manner. When the above treatment was applied to cholesterol the acetate was formed in quantitative yield.

Experimental

The melting points were taken in open Pyrex glass capillaries and are corrected for stem exposure. The rotation measurements were carried out in a 1-dm. semi-micro tube, with chloroform as the solvent, unless indicated otherwise. The ultraviolet spectra were measured in a quartz Beckman spectrophotometer, Model No. DU, and those of the more important compounds were later checked in a Cary self-recording instrument. Most of the infrared spectra were determined in Nujol mull in a Perkins–Elmer model 12-B single beam instrument, and in a few cases in the double beam self-recording model 21.

Tetrahydrojervin-11α-ol (22,26-imino-17,23-oxidojervane-3β,11α-diol) (II) was prepared by reduction of tetrahydrojervine with sodium and *n*-butanol as described by Jacobs and Huebner[2]; m.p. 283–285°, $[\alpha]^{27}D$ +39.5° (*c* 0.86), +47° (*c* 1.03 in absolute ethanol); lit.[2] m.p. 293–296°, $[\alpha]^{29}D$ +48.5° in 95% ethanol; λ_{max}^{nujol} 2.96, 3.25 μ, no bands in 6.0 region.

The **triacetate (IIa)** was prepared from 500 mg. of II with acetic anhydride (3 cc.) and pyridine (5 cc.) in the usual manner (room temperature, 20 hours). On recrystallization from aqueous methanol it formed platelets, m.p. 161–164°, while from hexane it crystallized in clusters of prisms, m.p. 175–178°, $[\alpha]^{23}D$ +69° (*c* 1.17); $\lambda_{max}^{chlf.}$ 5.79, 6.12, 8.03 μ, no absorption in 3.0 region.

(8) D. H. R. Barton and E. Miller, THIS JOURNAL, **72**, 1066 (1950).

(9) Of the stereoisomeric 5,6-dibromocholestanyl acetates, which would be most suitable for comparison with the diacetyljervine dibromides, only the 5α,6β-isomer has been rotationally characterized[8]; $\Delta[M]D$ for its formation from cholesteryl acetate is −97°.

Anal. Calcd. for $C_{33}H_{51}O_6N$ (557.7): C, 71.05; H, 9.22; 3 $COCH_3$, 23.1. Found: C, 71.03; H, 8.91; $COCH_3$, 18.4.[10]

For the preparation of the **N-acetate (IIb)** 300 mg. of II was dissolved in warm dry methanol (10 cc.) and acetic anhydride (0.1 cc.) was added after cooling. After standing for 4 hours at room temperature the mixture was worked up in the usual manner, and the product was recrystallized twice from acetone (141 mg.); m.p. 257–259°, $[\alpha]^{25}D$ +84° (*c* 0.98).[10]

Anal. Calcd. for $C_{29}H_{47}O_4N$ (473.7): C, 73.52; H, 10.00; $COCH_3$, 9.08. Found: C, 73.70; H, 10.04; $COCH_3$, 1.68.

N-Acetyltetrahydrojervone (22,26-Imino-17,23-oxidojervane-3,11-dione N-acetate) (III).—To a solution of N-acetyltetrahydrojervin-11α-ol (IIb, 120 mg.) in acetic acid (4 cc.) chromium trioxide (37 mg., 2.2 molar equivalents oxygen) in acetic acid (7.4 cc.) was gradually added in the course of 30 minutes. After standing for the same period the solvent was removed *in vacuo* and the neutral products were isolated in the usual way by chloroform extraction. Only traces of acids were present. Crystallization from ethanol yielded needles (76 mg.) of constant m.p. 267–271°, $[\alpha]^{26}D$ +27.5° (*c* 0.950), λ_{max}^{alc} 295 mμ (46).

Anal. Calcd. for $C_{29}H_{43}O_4N$ (469.6): C, 74.16; H, 9.23; N, 2.98. Found: C, 74.20; H, 8.97; N, 3.17.

The **mono-2,4-dinitrophenylhydrazone** prepared from the diketone with Brady's reagent after recrystallization from benzene–ethanol melted at 255–256°.

Anal. Calcd. for $C_{45}H_{47}O_7N_5$(649.8): C, 64.69; H, 7.29; N, 10.8. Found: C, 64.92; H, 7.82; N, 10.2.

For the preparation of the diketone from N-acetyltetrahydrojervine 84 mg. of the latter was oxidized with chromium trioxide (14.4 mg., 1.2 molar equivalents oxygen) as described above for IIb, m.p. 265–270°, undepressed by admixture with the specimen from IIb, $[\alpha]^{25}D$ +28° (*c* 0.510).

The dinitrophenylhydrazone had m.p. 255–256°, undepressed by above specimen.

Tetrahydrojervin-11β-ol (22,26-Imino-17,23-oxidojervan-3β,11β-diol) (IV).—To a solution of tetrahydrojervine (502 mg.) in tetrahydrofuran (17 cc.) lithium aluminum hydride (125 mg.) in the same solvent (3 cc.) was added with stirring. The solution gradually assumed gelatinous consistency. After 1.25 hours water was added till the evolution of hydrogen ceased. The mixture was diluted with ice-water and extracted 3 times with chloroform. The combined extracts were washed with dilute sodium hydroxide solution and water, and dried. The residue was recrystallized from aqueous ethanol (m.p. 171–175°) and then twice from ethyl acetate from which it formed large rhombohedra (203 mg.) melting at 252.5–255° (the product from the first recrystallization from ethyl acetate showed a double melting point, 170–180°, resolidification at 252–255°), $[\alpha]D$ +36° (*c* 0.916). Since the analysis was not quite satisfactory (probably due to the retention of ethyl acetate not removed by drying at 110°, as was indicated by the presence of a strong band at 5.75 μ in the subsequently determined infrared spectrum), the material was combined with the mother liquor residues, dissolved in chloroform and chromatographed on alumina (12 × 111 mm.). Continued washing with chloroform (8 × 40 cc.) initially removed crystalline fractions (A, 76 mg.) showing low melting points in the range 148–160°. The fractions obtained on further elution with chloroform (8 × 40 cc.) and then with similar amounts of chloroform containing 0.5, 1.5 and 5% methanol (fractions C, D and E, respectively) all melted in the range 240–250°, and on two recrystallizations from aqueous methanol yielded needles melting at 252–255° or slightly lower, in most cases after previous melting ~170° and resolidification. Fraction D was dried to constant weight at 138° (0.1 mm.) (weight loss 2.1%) and then melted at 246.5–248.5°, $[\alpha]^{21}D$ +25° (*c* 0.938); λ_{max}^{nujol} 2.84, 3.02 μ, and low band of unexplained origin at 6.12 μ.

Anal. Calcd. for $C_{27}H_{45}O_3N$ (431.6): C, 75.13; H, 10.51. Found: C, 75.20; H, 10.24.

Fraction A on recrystallization from methanol–ethyl acetate yielded first a small amount of crystalline material, m.p. 310–324°, which markedly depressed the melting point of tetrahydrojervin-11α-ol. On concentration of the mother liquor 76 mg. of needles, m.p. 160–163°, was obtained, which after two recrystallizations from aqueous methanol melted at 163–166°, unchanged after drying at 138° (0.1 mm.) (weight loss 4.9%). The infrared spectrum was substantially identical with that of fraction D, but the analysis showed a deficit in both C and H which could not be rationalized (C, 72.20; H, 9.62).

The remainder of fraction D was combined with fraction E (70 mg.) and acetylated with acetic anhydride in pyridine at room temperature. The product resisted all attempts at crystallization. It was lyophilized from benzene and dried to constant weight at 66° (0.1 mm.), $[\alpha]^{20}D$ +59° (*c* 0.77); λ_{max}^{nujol} 2.92, 5.77, 6.10, 8.08 μ.

Anal. Calcd. for $C_{31}H_{49}O_5N$ (515.7): 2$COCH_3$, 16.7. Found: $COCH_3$, 15.0.

The diacetate (51 mg., 0.099 mmole) was dissolved in acetic acid, and a solution of chromium trioxide in acetic acid containing 0.099 milliatom of active oxygen per cc. was added in small increments. Consumption of the reagent stopped after the addition of 1.3 cc. The product, isolated in the usual way, crystallized from aqueous ethanol (32 mg., m.p. 210–215°). After two recrystallizations it melted at 218–220° and did not depress the m.p. of an authentic sample of diacetyltetrahydrojervine (m.p. 218–220°). The identity with the latter was confirmed by the specific rotation $[\alpha]^{21}D$ −6.0° and the infrared spectrum.

Jervin-11β(?)-ol (22,26-Imino-17,23-oxido-5,13(17a)-jervadiene-3β,11β(?)-diol (V).—Jervine (1.5 g.) was reduced in tetrahydrofuran (55 cc.) with lithium aluminum hydride (400 mg.) as described above for tetrahydrojervine, except that the reaction was carried out under nitrogen. The residue from the sodium hydroxide and water-washed chloroform extract on trituration with acetone yielded 1.15 g. of crystalline material which was subjected to extraction with ether in a Soxhlet apparatus. Repeated recrystallization of the ether-insoluble residue (394 mg.) from methanol–acetone afforded prisms melting at 278–283°, $[\alpha]^{25}D$ −70° (*c* 0.922 in absolute ethanol); λ_{max}^{nujol} 2.99 μ, no distinct maxima in 4–6.5 μ region; no specific ultraviolet absorption.

Anal. Calcd. for $C_{27}H_{41}O_3N$(427.6): C, 75.82; H, 9.67. Found: C, 75.78; H, 9.58.

Acetylation of V (84 mg.) with acetic anhydride and pyridine (room temperature, 60 hours) yielded a sirup which was chromatographed on alumina (4 g.) in hexane–benzene (9:1). Elution was effected with the usual sequence of solvent mixtures. After the elution of a small crystalline fraction (needles, m.p. 185°) with benzene–ether (9:1), the remainder of the eluted material was recovered mostly in the benzene–ether (5:1, 1:1), ether and ether–acetone (9:1) eluates. All these fractions, when moistened with ether–pentane, gave prisms melting in the same range (207–218°). They were combined (62 mg.) and recrystallized twice from acetone–ether, yielding 30 mg. of the diacetate (Va); m.p. 213–217°, $[\alpha]^{25}D$ −36° (*c* 0.894).

Anal. Calcd. for $C_{31}H_{45}O_5N$(511.7): C, 72.75; H, 8.87; 2$COCH_3$, 16.8. Found: C, 73.24; H, 9.34; $COCH_3$, 12.1.

The ether-soluble fraction (591 mg.) of the reduced mixture yielded after two recrystallizations from acetone 192 mg. of dihydrojervine, m.p. 240–242°, undepressed by authentic material, $[\alpha]^{25}D$ −73° (*c* 1.03 in ethanol).

Anal. Calcd. for $C_{27}H_{41}O_3N$ (427.6): C, 75.82; H, 9.67. Found: C, 76.06; H, 9.31.

The identity with dihydrojervine was further confirmed by the preparation and analysis of the diacetate, m.p. 210–213°, $[\alpha]^{25}D$ −61° (*c* 0.847).

Diacetyljervine 5,6-Dibromides A and B.—Diacetyljervine (1.0 g.) was dissolved in acetic acid (10 cc.) containing potassium acetate (1 g.), and a standardized solution of bromine in acetic acid (90.6 mg. of Br per cc.) was added dropwise with shaking until no further decolorization was observed (4.4 cc.); the total uptake of bromine corresponded to 1.26 mole per mole of diacetyljervine. The solution was concentrated *in vacuo* to a small volume, diluted with water and extracted with chloroform. The combined extracts were washed with sodium bicarbonate solution and water, dried and evaporated to dryness. The residual sirup crystallized on addition of ethanol. The crude crystalline product was recrystallized from ethanol (824 mg.), and the

(10) Most acetylated jervine derivatives yield only a fraction of the N-acetyl group in the Kuhn-Roth acetyl determination; *cf.* O. Wintersteiner and M. Moore, THIS JOURNAL, **75**, 4938 (1953), p. 4940.

resulting crystals were digested with ether (30 cc.) at room temperature. The ether-insoluble residue (80 mg.), m.p. 158–159° on recrystallization from ethanol, gave shiny needles of dibromide A, m.p. 157–158°, $[\alpha]^{25}D$ −46° (*c* 1.196); λ_{max}^{ale} 250 mμ (19,500), 355 mμ (86).

Anal. Calcd. for $C_{31}H_{43}O_5NBr_2$ (669.5): C, 55.61; H, 6.47; Br, 23.9. Found: C, 56.59; H, 6.43; Br, 24.1.

The ether extract, was brought to dryness, and the residue was recrystallized from ethanol, yielding 615 mg. of dibromide B (fine needles), m.p. 146–147°, $[\alpha]^{24}D$ −68° (*c* 1.39); λ_{max}^{ale} 250 mμ (17,000), 355 mμ (60).

Anal. Calcd. for $C_{31}H_{43}O_5NBr_2$ (669.5): C, 55.61; H, 6.47; Br, 23.9. Found: C, 55.90; H, 6.10; Br, 23.8.

No appreciable changes in rotation were observed with either dibromides when their solutions in chloroform were allowed to stand at room temperature for 6 days.

Dibromide A (40 mg.) was debrominated by refluxing its solution in ethanol (5 cc.) with zinc dust (200 mg.) for one hour. On gradual addition of water needles (27 mg.) deposited which after recrystallization from 50% aqueous ethanol melted at 171–172°, and did not depress the melting point of an authentic sample of diacetyljervine (m.p. 170–171°).

The same result was obtained with dibromide B.

Jervine Dibromide Hydrobromide.—To a solution of jervine (100 mg.) in acetic acid (3 cc.) bromine in the same solvent (15.1 mg./cc.) was added dropwise at intervals over the course of 90 minutes. Addition was stopped when 3.1 cc. had been decolorized. On standing overnight, a crystalline precipitate (51 mg.) separated, which was recrystallized by dissolving it in hot methanol (15 cc.) and concentrating to 3 cc.; needles, m.p. 217–219°, $[\alpha]^{25}D$ −88° (*c* 0.328 in 50% aqueous ethanol); λ_{max}^{ale} 245 mμ (18,300); λ_{max}^{nujol} 3.03, 5.88, 6.17 μ.

Anal. Calcd. for $C_{27}H_{39}O_3NBr_2 \cdot HBr$ (667): Br, 36.0. Found: 34.6.

O-Acetyljervine.—A solution of jervine (500 mg.) in acetic acid (10 cc.) was refluxed for 18 hours. The solvent was removed *in vacuo*, and the dark-red residue was taken up in water. After alkalization with sodium hydroxide the solution was extracted with chloroform, and the extract was washed with water, partially decolorized by treatment with charcoal, and taken to dryness. The crystalline product obtained on the addition of ethanol to the residue (221 mg.) was recrystallized from acetone: needles, m.p. 277–279° $[\alpha]^{27}D$ −138° (*c* 0.54 in absolute ethanol); λ_{max}^{ale} 252 mμ (14,700), 360 mμ (53); λ_{max}^{nujol} 5.79, 5.89, 6.14, 7.98 μ.

Anal. Calcd. for $C_{29}H_{41}O_4N$ (467.7): C, 74.47; H, 8.84; $COCH_3$, 9.20. Found: C, 74.51; H, 8.74; $COCH_3$, 9.5.

On hydrolysis with 5% methanolic potassium hydroxide (refluxing 30 min.) pure jervine, m.p. 241–243°, $[\alpha]^{25}D$ −148° (*c* 1.03 in ethanol), was obtained in almost quantitative yield.

O-Acetyldihydrojervine.—Dihydrojervine was acetylated as described above for jervine. The melting point of the crude product (375 mg., 66% of theory) was 280–282°, and remained unchanged on recrystallization from methanol; $[\alpha]^{24}D$ −100° (*c* 0.948), −86° (*c* 0.548 in absolute ethanol); λ_{max}^{nujol} 5.80, 7.95.

Anal. Calcd. for $C_{29}H_{43}O_4N$ (469.7): C, 74.16; H, 9.23; $COCH_3$, 9.16. Found: C, 74.45; H, 9.10; $COCH_3$, 9.3.

Alkaline hydrolysis yielded pure dihydrojervine, m.p 242–244°, and N-acetylation in methanol diacetyldihydrojervine, m.p. 213–216°, $[\alpha]^{25}D$ −64° (*c* 1.30).

O-Acetyltetrahydrojervine prepared from tetrahydrojervine in the above manner (yield 49%) melted at 250–252° and showed $[\alpha]^{25}D$ −48° (*c* 0.851); λ_{max}^{nujol} 5.80, 7.96 μ.

Anal. Calcd. for $C_{29}H_{45}O_4N$ (471.7): C, 73.83; H, 9.62; $COCH_3$, 9.12. Found: C, 73.72; H, 9.57; $COCH_3$, 9.50.

Alkaline hydrolysis regenerated tetrahydrojervine, m.p. 212–214°.

Cholesterol (500 mg.) when acetylated under the above conditions gave 436 mg. of the pure acetate, m.p. 212–213.5°, by recrystallization of the crude product. With dihydrojervine and tetrahydrojervine shortening of the reflux period to 5 hours resulted in lower yields and less pure products. Attempts to O-acetylate these compounds with acetic acid at room temperature in the presence of catalytic amounts of perchloric acid failed on account of the fact that unacetylated bases (in contrast to their O-acetyl derivatives) form perchlorates insoluble in cold acetic acid, while heating such mixtures results in decomposition.

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NEW BRUNSWICK, NEW JERSEY

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Hydrazides of Some Pyridazonyl Substituted Acids

BY FREEMAN H. MCMILLAN, KENNETH A. KUN, CAROL B. MCMILLAN, BENJAMIN S. SCHWARTZ AND JOHN A. KING

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Hydrazides have been prepared of a group of eighteen aliphatic carboxylic acids each of which contains a 6-pyridazonyl-1-substituent. These hydrazides were prepared by the reaction of hydrazine with the corresponding ethyl esters which in turn were prepared by alkylation of the appropriately substituted 6-pyridazone with bromosubstituted esters. Some of the starting pyridazones are new and their preparation is described. Some of these hydrazides under the condition of our test caused stimulation of the growth of embryonic chick fibroblasts.

As part of our microbiological screening program certain pyridazonyl substituted aliphatic hydrazides were incorporated into tissue cultures using the techniques of Dulbecco.[1,2] There was evidence from preliminary studies that certain compounds in the series could stimulate the growth of embryonic chick fibroblasts. In order to obtain a quantitative measure of cell proliferation in tissue culture the method of Sanford[3] was used. A description of the microbiological procedures and results, with a discussion of the possible therapeutic implications, will be published elsewhere. The present paper describes the synthesis of these hydrazides and of intermediates necessary for their preparation.

In a previous communication[4] we have shown that 6-pyridazone and 3-methyl-6-pyridazone were easily alkylated with α-bromoacetic esters in the presence of sodium ethoxide in ethanol. Using the same general method we have prepared a number of pyridazonyl alkanoic esters by using a variety of bromosubstituted esters to effect the

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