of increasing electromobility these four major fractions were identified: (I) *free* homoserine resulting from *free* homoserine lactone; (II) "core" material together with ribonuclease (possibly containing methionine sulfoxide which is resistant to NCBr); (III) 25% (in terms of whole protein methionine) (theor.) of isolated *chemical tail peptide*, presumably a heptadecapeptide (in contrast to the enzymatically produced 20-residue S-peptide[6]), containing the original NH_2-terminal lysine and a C-terminal homoserine (lactone), (IV) *free* homoserine lactone, by cleavage of the methionyl-methionyl-lysine (29–30–31) sequence; its yield together with the *free* homoserine with which it equilibrates in aqueous solution approximates 50% (in terms of whole protein methionine.)

TABLE I

SURVEY OF THE NH_2-TERMINAL RESIDUES INVOLVED IN THE CLEAVAGE OF RIBONUCLEASE BY CYANOGEN BROMIDE

Fraction	Dinitrophenylation and Hydrolysis Products	NH_2-term. resid. Found[a]	Calc.	Numerical Position and Sequence of Methionine(s) Involved in Cleavage
Original mixture of reaction of cyanogen bromide with ribonuclease	Di-DNP-Lys	0.90	2.0	Met-Lys (Lys) 30 31 (1)
	DNP-Ser	0.85	2.0	Met-Ser 17 18 79 80
	DNP-HomoSer, DNP-HomoSer lactone	0.50	1.0	Met-*Met*-Lys 29 30 31
Electrophoretic fraction II: ribonuclease (METH-O) and modified "Core"	Di-DNP-Lys	0.75	2.0	Met-Lys (Lys) 30 31 (1)
	DNP-Ser	0.65	2.0	Met-Ser 17 18 79 80
Electrophoretic fraction III: chemical tail peptide (1–17)	Di-DNP-Lys	0.55[b]	1.0	(Orig. NH_2-terminal Lys)

[a] Corrections for losses during hydrolysis were made with authentic DNP-amino acids which were taken concomitantly through the entire procedure. [b] Hydrolysis time was 18 hours compared with 8 hours and 90% Di-DNP-Lys, *cf.* C. B. Anfinsen, *et al.*, *J. Biol. Chem.*, **207**, 201 (1954).

At this preliminary stage the question of glutamic acid occurring either in positions 18[4] or 11 (Fig. 1)[7] seems to be answered for *native* ribonuclease in favor of position 11[7] (i) by the absence of DNP-Glu,[8] (ii) by the presence of 0.65–0.85 of 2.0 DNP-Ser (Table I) and (iii) by the consistent ratio of Glu:Ala:Phe of 3:3:1 in the amino acid composition of Fraction III.[9]

A study of the reaction of cyanogen bromide in 0.3 *N* HCl with the common amino acids showed that, besides methionine, only cysteine,[10] but

(6) *Cf.* P. J. Vithayathil and F. M. Richards, *J. Biol. Chem.*, **235**, 2343 (1960).

(7) R. R. Redfield and C. B. Anfinsen, *J. Biol. Chem.*, **221**, 385 (1956); C. B. Afinsen, M. Sela and H. Tritch, *Arch. Biochem. Biophys.*, **65**, 156 (1956).

(8) Up to 0.3 of 1 residue of DNP-Asp is found in the original cleavage mixture and in Fraction II. An inherently labile aspartyl or asparaginyl residue in our opinion is rearranged and its amino group liberated concomitant with, or subsequent to, NCBr cleavage.

(9) The supplementary NCBr cleavage of the 20-residue S-peptide (ref. 6), is in progress.

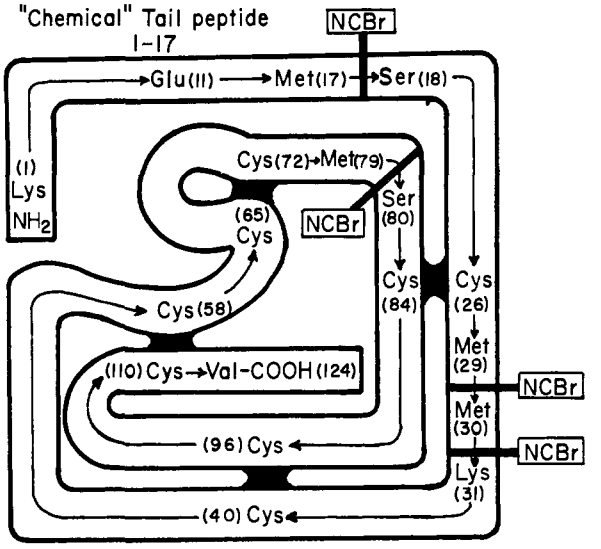

Fig. 1.—Topography of the cyanogen bromide cleavages of native ribonuclease [simplified diagrammatic "approximation" of D. H. Spackman, W. H. Stein and S. Moore, *J. Biol. Chem.*, **235**, 656 (1960)], with Glu (11) and Ser (18) arranged according to C. B. Anfinsen and R. R. Redfield, *Adv. in Protein Chem.*, **14**, 255 (1959).

neither cystine, tyrosine nor tryptophan, reacted. Cleavage of the α-chain of human hemoglobin containing two methionines[11] has been observed and led to three fractions on a sephadex-G25 column.[12,13]

(10) *Cf.* J. M. Swan, "Current Trends in Heterocyclic Chemistry," Editors A. Albert, G. M. Badger, C. W. Shoppee, Academic Press, Inc., New York, N. Y., 1958, p. 65.

(11) R. J. Hill and W. Konigsberg, *J. Biol. Chem.*, **235**, PC21 (1960); *cf.* G. Braunitzer *et. al.*, *Hoppe-Seyler's Z. physiol Chem.*, **320**, 283 (1960).

(12) The reaction of hemoglobin with cyanogen chloride has been studied previously in another connection without suspicion of cleavage: W. N. Aldridge, *Biochem. J.*, **48**, 271 (1950).

(13) W. Konigsberg and R. J. Hill, personal communication.

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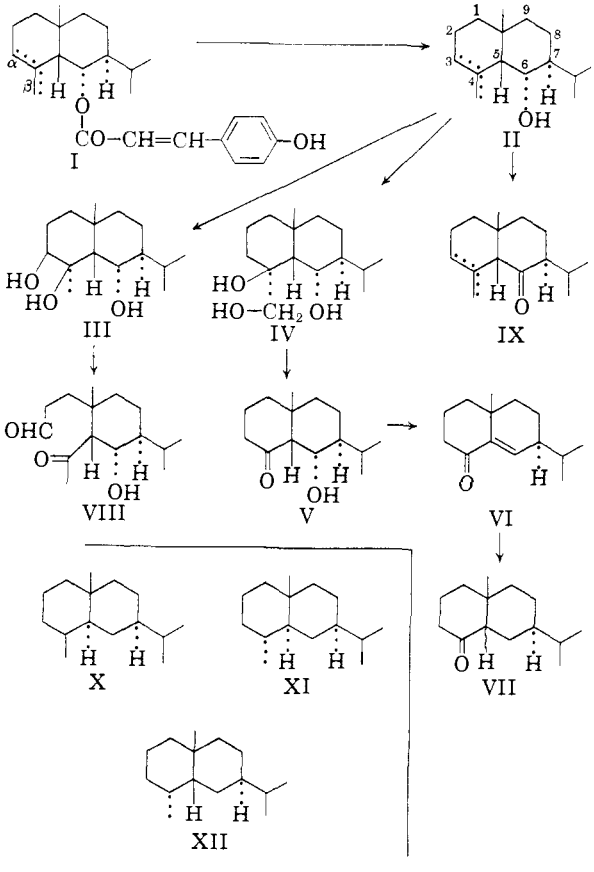
α- AND β-VERBESINOL. SESQUITERPENE ALCOHOLS OF THE *cis*-DECALIN SERIES

Sir:

Continuous petroleum ether extraction of the freeze-dried root of *Verbesina virginica* L. ("ice plant" or "crownbeard") affords up to 4% of a mixture of two isomeric sesquiterpene esters (I), m.p. 116–122°, $[\alpha]^{16}D$ +49°, λ_{max}^{EtOH} 230, 314 mμ (ϵ 8,300, 18,200), $\lambda_{max}^{0.1N\ NaOH}$ 313, 367 mμ (ϵ 2,500, 26,000).[1] The methyl ethers of I, formed readily by treatment of I with dimethyl sulfate, gave rise to *p*-hydroxycinnamic acid under pyrolytic conditions. Dehydrogenation (Pd–C) afforded eudalene and β-(*p*-methoxyphenyl)-propionic acid.

(1) All compounds described gave satisfactory analytical data. Optical rotations were measured using chloroform as the solvent.

Although I is resistant to base hydrolysis, two successive reductions with $LiAlH_4$ sufficed to cleave it to a mixture of α- and β-verbesinol (II), b.p. 82–83° (0.2 mm.), $[\alpha]^{22}_D \pm 0°$. The mixture could not be resolved into pure α- and β-isomers but a pure sample of the former was obtained by the reductive cleavage of dihydro I (described below), $[\alpha]^{21}_D -15.3°$. Hydroxylation of II with OsO_4 or $KMnO_4$ gave a mixture of triols (III and IV). Chromatography provided α-verbesintriol (III), m.p. 149–151°, $[\alpha]^{22}_D +16.0°$ and β-verbesintriol (IV), m.p. 146–147°, $[\alpha]^{22}_D -11.7°$. Pure IV was also obtained directly from I. Lead tetraacetate cleavage of IV gave, in addition to formaldehyde, a ketol (V), m.p. 49.5–50.0°, $[\alpha]^{22}_D -73.2°$ which was shown by its base-catalyzed dehydration to be a β-ketol. Similar cleavage of III gave liquid product (VIII) with functionality established as –OH, –CHO and $-COCH_3$ by use of the usual tests and spectroscopic data. This substance was not studied further.



The methoxide ion catalyzed dehydration of V gave VI, evaporatively distilled at 55° (0.01 mm.), $[\alpha]^{21}_D +34.8°$, λ^{EtOH}_{max} 242 mμ. Hydrogenation of VI to the saturated ketone (VII) and then reduction ($LiAlH_4$), dehydration and dehydrogenation afforded 2-isopropylnaphthalene, identified by comparison with an authentic sample.

Oxidation[2] of II with CrO_3 at 30° gave a mixture of α- and β-verbesinones (IX), b.p. 78° (0.1 mm.), $[\alpha]^{22}_D +42.3°$. Hydrogenation of IX afforded dihydroverbesinone which, upon bromination, gave a dibromo compound, m.p. 160.5–161.0°, $[\alpha]^{22}_D \pm 0°$.

These data establish the gross structure of II (β-isomer) as being stereomeric with that of junenol.[3] As is shown below, the configurations at C-7 and C-10 are identical with those of the corresponding atoms in junenol. The non-identity of the bromination product of dihydroverbesinone with that derived from dihydrojunenone can be attributed only to a configurational difference at C-4 in these two hydrogenation products. This suggests that the methyl group of dihydro IX is oriented α, a consequence of hydrogenation from the β side of the molecule. This would be the expected stereochemical course of reduction if the rings of IX were *cis*-fused but not if they possessed a *trans* geometry.

The configurations of carbon atoms 5, 7 and 10 (and consequent verification of the existence of a *cis*-fusion) were established by hydrogenolysis experiments. Reduction (very slow) of "purified" dihydroeudesmol, m.p. 85–86°, by means of Pd–C in ethyl alcohol gave 85% of X and 15% of XI (vapor–liquid chromatography). Similar reduction of II (very fast) afforded 27% of X and 73% of XI. Reduction of I (slow) gave 14% of X, 18% of XI and 68% of a new hydrocarbon which, by exclusion, must be XII. This interesting epimerization at C-5 in the reduction of II, but not I, will be fully discussed in the complete manuscript. It is clear, however, that the hydrogenation of the double bond of I precedes hydrogenolysis of the ester function from the fact that the interruption of the process after the absorption of one mole of hydrogen affords a dihydro derivative of I in which only the cinnamate double bond has been reduced. The α-isomer was obtained crystalline, m.p. 134–136°. This order of reduction as well as the relative ratios of X, XI and XII obtained in the hydrogenolysis experiments are steric manifestations of the *cis* juncture in I.

The configuration of C-6 in II must be α (equatorial) as prolonged base treatment of II did not induce epimerization. This conclusion is supported by the very slow rate at which II was oxidized to IX. The fact that the same triol (IV) was formed by the OsO_4 oxidation of I or II proves that they have identical configurations at C-6 and thus the total stereochemistry of these substances is as shown.

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