difficult to rationalize in terms of theoretical models based on electrostatic theory.

The specific solubilizing effects observed for arginine hydrochloride, histidine hydrochloride, and proline reflect the ability of these amino acids to specifically decrease the activity coefficients of certain other amino acids. These changes in activity coefficients must reflect specific interactions between pairs of amino acids, since the observed changes in activity coefficients cannot be explained in terms of nonspecific medium effects. It is realized that the change in activity coefficient of one amino acid in the presence of another amino acid is complicated by ionic as well as apolar interactions. Therefore, the linear dependence of the solubility of one amino acid on the concentration of another amino acid should only be taken as circumstantial evidence for the formation of a 1:1 complex. Since the apparent value for the dissociation constant estimated from solubility data reflects the strength of the interaction even if no complex actually forms,[17] we have chosen to use this parameter to characterize each interaction.

It is not surprising that histidine hydrochloride and arginine hydrochloride appear to interact readily with several other amino acids, since imidazole hydrochloride and guanidine hydrochloride are effective denaturing agents of proteins.[18] Perhaps externally added guanidine hydrochloride or imidazole hydrochloride disrupts specific interactions between amino acid side chains by binding directly to one or more of the interacting side chains.

Robinson and Jencks[19] explain the increased solubility of acetyltetraglycine ethyl ester in guanidine hydrochloride solutions by assuming a 1:1 complex forms between guanidine hydrochloride and acetyltetraglycine ethyl ester. The apparent dissociation constant of this complex is 1.1 *M* and is similar to the values we report for the complexes of arginine hydrochloride. It should be noted, however, that polyfunctional hydrogen bonding is probably responsible for these interactions with guanidine hydrochloride, whereas the interactions listed in Table I cannot be attributed entirely to hydrogen bonding. Arginine hydrochloride is most effective in increasing the solubility of tryptophan, tyrosine, phenylalanine, and asparagine. Nozaki and Tanford[11] studied the increase in solubility of 11 amino acids in urea solution. Their results indicate that urea is also most effective in increasing the solubility of tryptophan, tyrosine, phenylalanine, and asparagine.

Although the apparent dissociation constants listed for the complexes in Table I are large these interactions might be significant in determining the tertiary structure of proteins. Two amino acid side chains forming a complex with a dissociation constant of 2 *M* would be associated about 90% of the time when on the same protein molecule, assuming that on a protein molecule the effective concentration of one residue with respect to another residue is 20 *M*.

Further studies of the colligative properties of solutions of pairs of amino acids and peptides are under way in order to further assess the possible contributions of specific interactions between amino acid side chains to the tertiary structure of proteins.

(17) The free energy change (ΔF_{tr}) for transferring amino acid B from pure water to another medium at the same molar concentration of amino acid B is simply $-RT \ln([B^0]/K_S)$, where K_S and $[B^0]$ represent the molar solubility of amino acid B in water and the other medium respectively. Since the solubility data are represented by eq 1, even if no complex actually forms, $\Delta F_{tr} = -RT \ln[([A^0]/K_S + K_D) + 1]$. When $K_D \gg K_S$ this free energy change may be approximated by $-RT \ln[([A^0]/K_D) + 1]$.

(18) J. A. Gordon and W. P. Jencks, *Biochemistry*, **2**, 47 (1963).

(19) D. R. Robinson and W. P. Jencks, *J. Am. Chem. Soc.*, **87**, 2462 (1965).

Communications to the Editor

The Structure of a C_{19}-Diketone Derived from Ryanodine[1]

Sir:

Ryanodine, $C_{25}H_{35}O_9N$, isolated from *Ryania speciosa* Vahl,[2] is of interest because of its high oxygenation and the lack of structural analogies among natural products as well as its extreme and exceptional toxicity[2a,3] and insecticidal action.[2a,4] Hydrolysis leads to pyrrole-α-carboxylic acid and ryanodol, $C_{20}H_{32}O_8$.[2] Structural assignments for ryanodine and ryanodol have been based in part on acid-rearranged products and C_4–C_8 fragments resulting from the action of alkali on these products.[5]

We have degraded ryanodol to a C_{19} compound to which a unique structural assignment can be made. Ryanodol,[6,7] mp 247°, $[\alpha]$ +18°, with excess periodate[8]

(1) This work was sponsored by the U. S. Army Chemical Corps, the U. S. Army Research Office, Durham, and the National Institutes of Health, U. S. Public Health Service. It was presented in part at the Tenth Organic Chemistry Conference, U. S. Army Natick Laboratories, Oct 4, 1966.

(2) (a) E. F. Rogers, F. R. Koniuszy, J. Shavel, Jr., and K. Folkers, *J. Am. Chem. Soc.*, 70, 3086 (1948); (b) R. B. Kelly, D. J. Whittingham, and K. Wiesner, *Chem. Ind.* (London), 857 (1952), (c) we are greatly indebted to Dr. E. F. Rogers (Merck Sharp and Dohme) for information on his preliminary investigations and for various samples.

(3) W. L. Haslett and D. J. Jenden, *J. Cellular Comp. Physiol.*, **57**, 123 (1961).

(4) K. D. Arbuthnot, *J. Econ. Entomol.*, **51**, 562 (1958).

(5) See K. Wiesner, Z. Valenta, and J. A. Findlay, *Tetrahedron Letters*, 221 (1967), for the most recent structural postulate and references to previous proposals. Consideration of these structures and of our data will be made in a future publication. Also, after this paper was submitted for publication, a structure for ryanodol *p*-bromobenzyl ether based on X-ray diffraction studies appeared by S. N. Srivastava and M. Przybylska, *Can. J. Chem.*, **46**, 795 (1968).

(6) The assistance of Dr. K. Folkers and Merck Sharp and Dohme in securing starting material was of inestimable value.

(7) Satisfactory elementary analyses were obtained for all compounds reported; ultraviolet spectra were taken in ethanol and are reported in $m\mu$ (ϵ); infrared spectra as KBr wafers are reported in cm^{-1}

Table I. Selected Nmr Absorptions[a] of Ryanodol Degradation Products

Compd	H OR	H C H C C=O	CH_3 C	CH_3 C	H_3C CH_3 C H	CH_3 CH
Ib	4.0 (d, 11)	2.8 (q, 18)	1.7	1.2	1.25 (d, 6)	1.0 (d, 7)
II	4.3 (d, 2)	3.0 (q, 18)	1.7	1.6	1.2 (q, 6)	1.1 (d, 7)
IIIa	4.2 (d, 2)	2.95 (q, 18)	1.1 (d, 6)[b]	1.55	1.05 (q, 6)	0.9 (d, 7)
IIIb	4.3 (d, 2)	3.05 (q, 18)	1.2 (d, 7)[b]	1.7	1.1 (q, 6)	1.0 (d, 7)
IV	4.5 (d, 2)	3.15 (q, 18)	1.2 (d, 7)[b]	1.7	1.2 (q, 6)	1.1 (d, 7)
Vb	5.4 (d, 2.5)	2.85 (q, 18)	2.05[c]	1.6		1.1 (d, 7)
VIb	4.0 (m)	2.85 (q, 18)		1.6		1.1 (d, 7)
VIII		2.9 (q, 18)		1.35		1.1 (d, 7)
IX		2.9 (q, 18)		1.5		1.0 (d, 7)
X		2.9 (q, 18)		1.3		1.0 (d, 7)
XI		2.6 (q, 18)		1.25		1.0 (m)
XIII		2.85 (q, 18)		1.66		2.35[d]

[a] δ values, followed in parentheses by multiplicity and coupling constant in hertz. [b] Secondary methyl in reduced compound. [c] Acetyl. [d] Aromatic methyl.

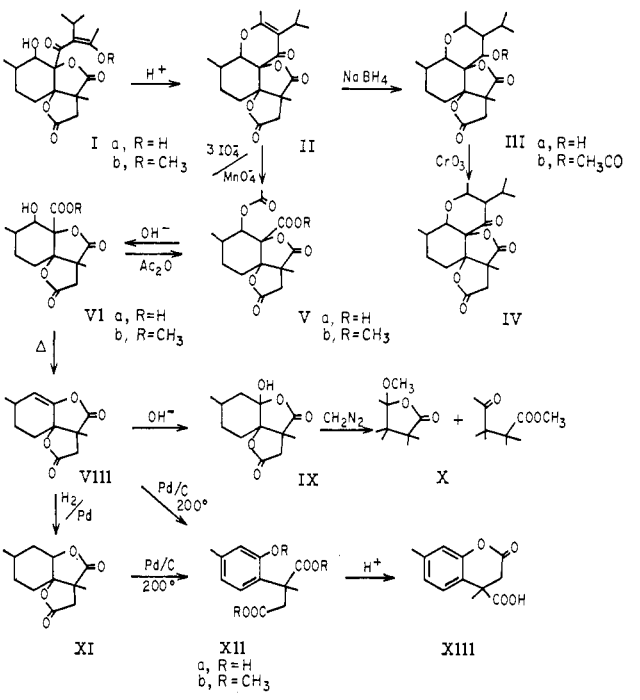
(3-mol consumption), gave $C_{20}H_{28}O_9$ (isolated as its Sr salt) which is easily hydrolyzed to the acidic product $C_{19}H_{26}O_7$ (Ia) [mp 223°; $[\alpha]$ +125°; λ_{max} 278 (10,000), $\lambda_{max}^{OH^-}$ 273 (12,500), 325 (5600); ν 3473, 1761, 1723, 1672, 1604]. The C_{19} compound Ia and its methylation (diazomethane) product Ib [mp 180°; λ_{max} 279 (9150), unchanged in alkali; ν 3476, 1764, 1732, 1694, 1630] have all the characteristics of a 1,3-diketone and its enol ether, respectively. They contain two lactones (ir), one secondary hydroxyl, one secondary methyl, one tertiary methyl, one vinyl methyl, and one isopropyl group (nmr, Table I).

With aqueous–ethanolic hydrochloric acid, Ia furnished the anhydro derivative $C_{19}H_{24}O_6$ (II) [mp 205°; λ_{max} 272 (10,000); ν 1790, 1739, 1695, 1638] whose spectral data require formation of an internal enol ether. II was reduced with borohydride to the tetrahydro derivative $C_{19}H_{28}O_6$ (IIIa) (mp 191°; $[\alpha]$ +63°; ν 3420, 1793, 1707). The doublet in the absorption of the proton on the new secondary carbinol carbon in IIIa (δ 3.7) and in its acetylated derivative IIIb (δ 5.3) requires one proton only on the adjacent carbon; IIIa was oxidized with chromium trioxide to the ketone $C_{19}H_{26}O_6$ (IV) [mp 160°; ν 1791, 1729].

A 3-mol periodate–permanganate oxidation[9] of enone II led to isobutyric acid and the acid $C_{15}H_{18}O_8$ (Va) (mp 279°; pK = 3.8; ν 3100, 1782, 1784, 1704), possessing an acetylated *sec*-hydroxyl and two γ-lactones. From its high acidity, Va must be an α-oxygenated acid. Hydrolysis of Va yielded the hydroxy acid $C_{13}H_{16}O_7$ (VIa) (mp 185°; pK = 3.3; ν 3450, 1781, 1760, 1705) whose methyl ester VIb (mp 194°; $[\alpha]$ −3°; ν 3300, 1798, 1768, 1735) was reacetylated to Vb (mp 184°; ν 1789, 1753, 1746. Lithium aluminum hydride reduction of VIb furnished the polyol $C_{13}H_{26}O_6$ (VII), mp 117°.

On heating, VIa evolved carbon dioxide, forming the enol lactone $C_{12}H_{14}O_4$ (VIII) (mp 96°; ν 1807, 1775; δ 5.65, vinyl H, d) which was hydrolyzed to the lactol $C_{12}H_{16}O_5$ (IX) [mp 151°; pK = 6.4 (50% EtOH); ν 3410, 1794, 1773]. Both VIII and IX consumed 2 mol of alkali; on acidification, IX was regenerated. Methylation of IX gave a mixture of keto and pseudo esters X [ν 1775, 1735, 1712 ($CHCl_3$); δ 3.6, OCH_3]. Hydrogenation of VIII afforded a mixture of epimeric dilactones XI. From its nmr absorption (δ 4.6, m), One of the two new protons in XI must be on an acyloxylated carbon.

Aromatization (di-*n*-hexyl ether, 30% Pd–C, 200°) of VIII and XI and hydrolysis yielded the succinic acid XIIa, which was methylated to an oil whose mass spectrum supports structure XIIb. XIIa with acid gave the aromatic acid $C_{12}H_{12}O_4$ (XIII) [mp 107°; $[\alpha]$ −62°; λ_{max} 270 (1290), 279 (1340); $\lambda_{max}^{OH^-}$ 246 (6700), 296 (3500); yield, VIII → XIII, 70%] to which structure *l*-4,7-dimethyl-4-carboxydihydrocoumarin was assigned and proved by synthesis: *m*-cresol with ethyl acetoacetate gave 4,7-dimethylcoumarin,[10] and addition



only for the hydroxyl and carbonyl regions; nmr spectra were taken at 60 Mc in $CDCl_3$ with internal TMS (δ 0); optical rotations were observed on 0.3–1.0% solutions in methanol at 23° with the sodium D line.

(8) Initial experiments by S. Masamune, Ph.D. Thesis, University of California, Berkeley.

(9) (a) R. U. Lemieux and E. V. Rudloff, *Can. J. Chem.*, **33**, 1701 (1955); (b) E. V. Rudloff, *ibid.*, **34**, 1413 (1956).

(10) K. Fries and W. Klostermann, *Ber.*, **39**, 871 (1906).

of HCN[11] furnished *dl*-4,7-dimethyl-4-carboxydihydrocoumarin, mp 105° after sublimation, identical with XIII in nmr, uv, and ir spectra and thin layer and gas chromatographic behavior.

Thus, the C_{12}-compound XIII, together with degradative fragments carbon dioxide, acetic acid, and isobutyric acid, represent all 19 carbon atoms of the C_{19}-diketone Ia. Consideration of the above evidence allows reconstruction only of structure Ia, as follows. The carbon skeleton of XIII is present in the enol lactone VIII which results from a decarboxylative dehydration of VIa. VIa and acetic acid are the hydrolysis products from Va. Va and isobutyric acid result from hydroxylation of the enone II and cleavage of the glycol and derived α-diketone. The enone II in turn is the internal enol ether of the 1,3-diketone Ia.

(11) J. Bredt and J. Kallen, *Ann.*, **293**, 363 (1896).

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Received March 1, 1968

The Structure of a C_{19} Acid Derived from Ryanodine[1]

Sir:

Periodate oxidation of ryanodol, the alcohol moiety of the pyrrole-α-carboxylic ester ryanodine,[2] furnishes two acidic products in nearly quantitative yield. One is the previously reported C_{19} 1,3-diketone[3] (50%) and the other is a C_{19} carboxylic acid (40%) to which we assign structure Ia on the basis of the following evidence.

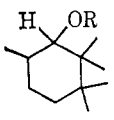
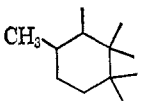
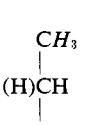
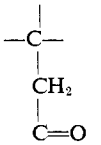
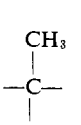
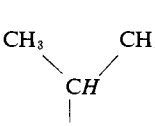
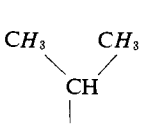
The acid[4] $C_{19}H_{28}O_8$ (Ia) (mp 163°; pK_a = 2.8; ν 3500–3300, 2700–2300, 1740–1715) and its methyl ester (Ib) (mp 139°; ν 3500, 1755, 1730, 1716) contain one secondary and one tertiary methyl, one isopropyl, one ethyl, one methylene between completely substituted carbons, and one proton on an acyloxylated carbon (nmr, Table I). Compound Ib further contains one hydroxyl (tritium exchange) and on oxidation (MnO_4^- or CrO_3) gives propionic, acetic, and isobutyric acids. The propionic acid originated from an ethyl ketone (shown as its hemiketal in Ia,b based on ir absorption), as shown by the nmr absorption pattern in which the methylene adjacent to the methyl appears as a quartet, δ 2.6. The high acidity of Ia indicates oxygen substitution on the α carbon, and this oxygen function was shown to be a (potential) hydroxyl by lithium aluminum hydride reduction to a polyol which on periodate oxidation gave formaldehyde.

The isopropyl group in Ia was proved to be present as an isobutyrate ester by the following: (1) lithium aluminum hydride reduction gave isobutyl alcohol; (2) both dilute alkaline hydrolysis and pyrolysis gave quantitatively isobutyric acid and carbon dioxide. The relative positions of the carboxyl and isobutyryloxy groups are assigned as shown in Ia to allow for their facile, concomitant elimination.

Ib with *t*-butoxide at 80° furnished quantitatively an acidic product, $C_{19}H_{26}O_7$ (II) (mp 237°; pK_a' = 5.4; λ_{max} 266 (7800), 305 (1900); $\lambda_{max}^{OH^-}$ 303 (16,000); ν 3400, 3300, 1730, 1706, 1660, 1619). Compound II, which is oxidized in air in the light, is a 1,3-diketone. Methylation (diazomethane) gave a mixture of isomeric methyl enol ethers, $C_{20}H_{28}O_7$, separated by crystallization into IIIa (mp 267°; λ_{max} 259 (9200); ν^{CHCl_3} 3500, 1728, 1682, 1613) and IIIb (mp 197°; λ_{max} 256 (7700); ν^{CHCl_3} 3500, 1723, 1666, 1618). Hydrogenation (1 mol) of II gave the β-hydroxy ketone $C_{19}H_{28}O_7$ (IV) (mp 243°; ν 3555, 3420, 1755, 1736, 1721). The nmr of IV shows two hydroxyl protons (deuterium exchange). The methylene next to the ketone carbonyl in II remains a singlet in IV, showing that the other carbonyl had been reduced. Appearance of a doublet at δ 3.9 (J = 4 Hz) in IV supports the introduction of a proton on a secondary alcohol carbon. IV in acidic aqueous ethanol undergoes dehydration to α,β-unsaturated ketone $C_{19}H_{26}O_6$ (V) (mp 260°; λ_{max} 238 (5800), 325–340 (60); ν 3600, 1752, 1710, 1645), with a new vinyl methyl at δ 1.8 coupled to the new vinyl proton at 6.25.

The 1,3-diketone II rapidly consumes 2 mol of

Table I. Selected Nmr Absorptions[a] of the C_{19} Acid and Its Derived Products

Compd							
Ib	5.3 (d, 9)	0.85 (d, 6)	1.0 (t, 6)	2.8 (q, 18)[b]	1.3	2.5 (sep, 6)	1.1 (q, 6)
II	5.4 (d, 10)	0.9 (d, 6)	1.2	2.8 (s)	1.4	2.6 (sep, 6)	1.2 (q, 6)
IIIa	5.3 (d, 10)	0.9 (d, 6)	1.8	2.15 (s)	1.25	2.6 (sep, 6)	1.2 (q, 7)
IIIb	5.3 (d, 10)	0.9 (d, 6)	1.8	2.6 (s)	1.2	2.6 (sep, 6)	1.2 (q, 7)
IV	5.4 (d, 10)	0.9 (d, 6)	1.1 (d, 6)	2.8 (s)	1.2	2.5 (sep, 6)	1.1 (q, 6)
V	5.3 (d, 10)	0.9 (d, 6)	1.8 (d, 2)[c]	2.8 (s)	1.25	2.6 (sep, 6)	1.2 (d, 6)
VI	5.2 (d, 11)	0.8 (d, 6)	2.25[d]	2.8 (s)	1.3	2.4 (sep, 6)	1.2 (d, 6)

[a] δ values, followed in parentheses by multiplicity and coupling constants in hertz. [b] The ketonic carbonyl exists as a hemiketal in this compound. [c] This is now a vinyl methyl coupled to the vinyl proton at δ 6.25 as a quartet, J = 2 Hz; intensity ratio 1:3:3:1. [d] Acetyl methyl in this compound.

(1) This work was sponsored by the U. S. Army Research Office, Durham. It was reported in part at the Tenth Organic Chemistry Conference, U. S. Army Natick Laboratories, Oct 4, 1966.

(2) Pertinent citations on ryanodine and its chemistry are given in ref 3.

(3) U. Hollstein and H. Rapoport, *J. Am. Chem. Soc.*, **90**, 3864 (1968).

(4) Satisfactory elemental analyses and spectral data were obtained for all compounds reported; ultraviolet spectra were taken in ethanol and are reported in mμ (ϵ); infrared spectra (as KBr wafers unless otherwise specified) are reported in cm^{-1} only for the hydroxyl and carbonyl regions; nmr spectra were taken at 60 and 100 Mc in $CDCl_3$ with internal TMS (δ 0).