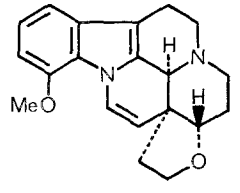
4.46, 3.79, 3.90, 4.13, 414.), λ_{min} (MeOH) 230 and 312 nm (lg ε 4.35 and 4.06). The 100 MHz ^{1}H-NMR-spectrum in benzene – d_6 reveals a close similarity to that of cuanzine[2], an alkaloid co-occuring in the same plant and containing the vincamine skeleton with an additional tetrahydrofurane ring (**2**). In fact, **1** exhibits, beside an 1,2,3-hydrogen aromatic substitution pattern at δ 7.21 (dd, J_{ortho} 7.5, J_2 2 Hz), 7.09 (t, J_{ortho} 7.5 Hz), 6.52 (dd, J_1 7.5, J_2 2 Hz) and a methoxy group at δ 3.42, the signals of four protons at δ 4.33 (1H, bs), δ 3.63 (1H, dd, J_1 12, J_2 7 Hz) and δ 3.98–3.75 (2H, m), having the same pattern as the C-21, C-15 and C-18 protons of **2**, respectively. In comparison to cuanzine, the signals of the AB system due to the protons at C-17, of the carbomethoxy group and of the hydroxyl function, are missing. The absence of the two latter functional groups is confirmed by the IR spectrum which lacks of any CO and OH absorption. In addition, the ^{1}H-NMR-spectrum exhibits the presence of an AB system (δ_A 7.66, δ_B 5.05, J_{AB} 8 Hz) attributable to two olefinic protons, one of which strongly deshielded.

The above spectroscopic data and the mass spectrum, which contains only one important peak at *m/e* 237 (100%, ion *a*), are consistent with structure **1** for the new alkaloid (decarbomethoxy apocuanzine), the cis C/D ring fusion being deduced from the lack of Bohlmann bands in the 2800–2730 cm^{-1} region of the IR-spectrum and from the downfield resonance of the C-21 proton.



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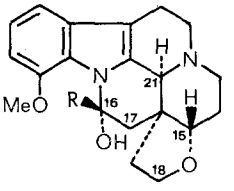
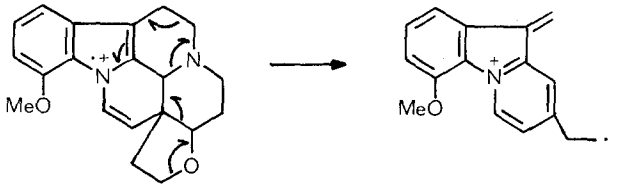
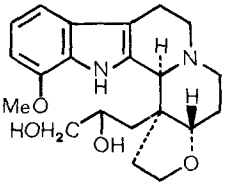


2 R = CO_2Me
3 R = H



a



4

Chemical confirmation of this supposition could be provided by correlation with cuanzine. Refluxing **1** in acetic acid yields the hydroxyderivative **3**, $C_{20}H_{24}N_2O_3$, M^+ = 340, OH stretching band at 3560–3440 cm^{-1} in the IR-spectrum, UV-maxima (MeOH) at 225, 270, 283 (infl.) and 292 nm (lg ε 4.60, 3.95, 3.86 and 3.73) characteristic of a methoxyindole chromophore.

In the ^{1}H-NMR-spectrum (100 MHz, $CDCl_3$) **3** exhibits, beside the C-15 proton at δ 5.47 (dd, J_1 10, J_2 7 Hz) and the C-21 proton at δ 4.25, an ABX system (δ_A 1.92, δ_B 2.56, δ^X 6.17, J_{AB} 15, J^{AX} 5, J^{BX} 2 Hz) which could be assigned to the geminal protons at C-17 and to an equatorial proton at C-16. Irradiation of the X proton transforms the signals at δ 1.92 and 2.56 into two doublets, the former displaying a slight broadening due to a long range W coupling with one of the protons at C-19 resonating at ca. 2.7 ppm. These data can be readily rationalized if the proton at C-17 resonating at δ 1.92 is placed in a β-axial position and the hydroxyl function at C-16 possesses an α-axial orientation.

Periodic acid cleavage of the diol **4** coming from the $NaBH_4$ reduction of cuanzine[2] yields the same hydroxyderivative **3**, thus establishing for the new alkaloid the absolute configuration shown in structure **1**.

Riassunto. Dalla corteccia della radice della *Voacanga chalotiana* è stata isolata la decarbometossi apocuanzina (**1**), un nuovo alcaloide indolico la cui struttura è stata definita per via spettroscopica e mediante correlazione chimica con la cuanzina (**2**).

Résumé. On a extrait de l'écorce des racines de *Voacanga chalotiana* un nouvel alcaloïde indolique, la décarbométhoxyapocuanzine. L'étude de son spectre de RMN et la corrélation chimique avec la cuanzine (**2**) permettent d'attribuer à cet alcaloïde la structure **1**.

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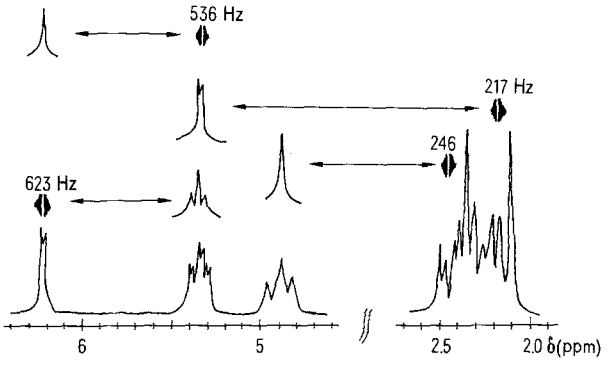
Chemical Behavior of Conjugated Polyenoic Acids toward Sulfuric Acid. Acid-Catalyzed Cyclization and Successive Rearrangement of *Trans*-β-Ionylidene Crotonic Acid

In connection with our program[1] aiming at the elucidation of the chemical behavior of conjugated polyenoic acids, especially of vitamin A acid (I) homologues, toward H_2SO_4, we describe here the first example on the title reaction of the conjugated tetraenoic acid.

Methods and materials. Treatment of *trans*-β-ionylidene crotonic acid (II)[2] in $CHCl_3$ with 80–90% H_2SO_4 at room temperature for a few min, and extraction of the diluted aqueous acidic layer with ether, led to the almost exclusive formation of the 2 major products. Because of the great



NMR (100 MHz) spectrum of the compound A (III)

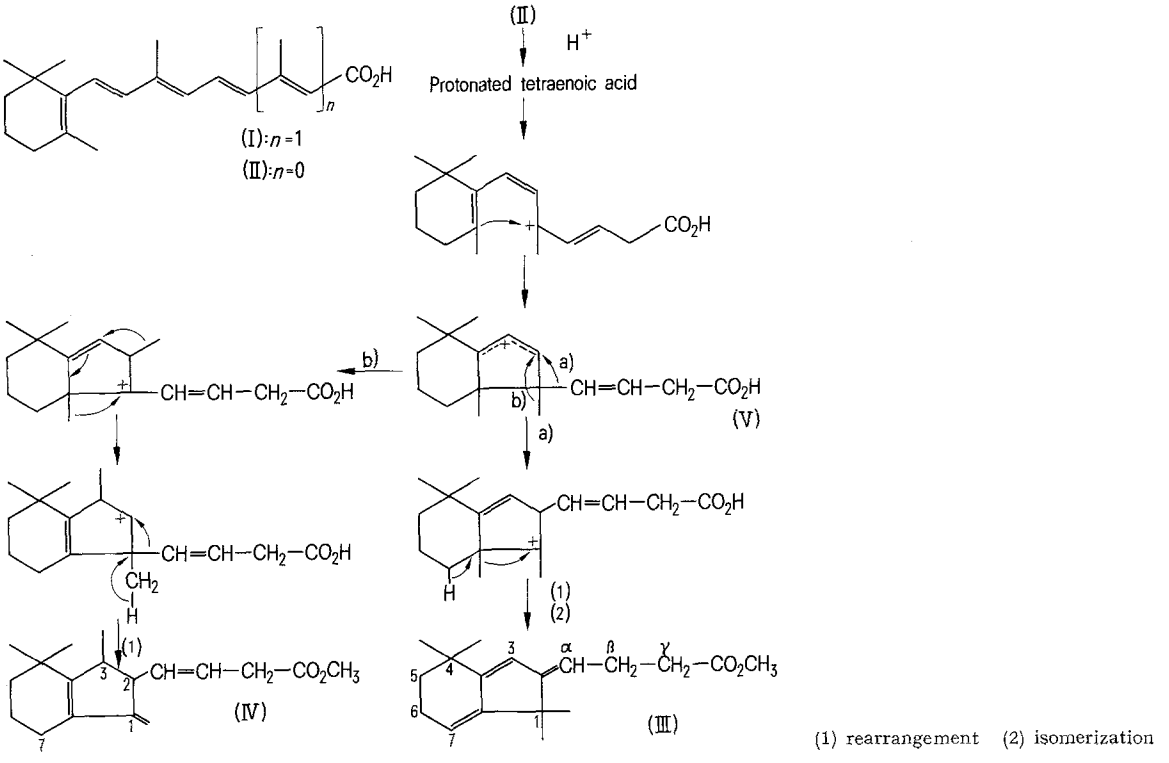
difficulty for obtaining an effective separation[3], the reaction mixture was methylated with diazomethane prior to the Pglc separation[4]. The compounds A and B, the corresponding methyl esters of the genuine components, were finally obtained in a pure state.

Compound A. $C_{18}H_{26}O_2$; mp., ca. 30°; R_t 10.5 min[4]; UV (nm)[5], 284; IR (cm^{-1}), [CCl_4] 1741 and 1165 (saturated aliphatic ester), 1642 and 1623 (C = C), and [CS_2] 1739, 1640, 1625, 1165, and 812 (trisubstituted alkene). No indication of the *trans*-CH=CH-group; NMR (δ ppm)[5], 0.99 and 1.11 (*s* and 6H each, 4 CH_3), 1.45 (2H, CH_2), 2.10–2.65 (*m*, 6H, C-6-, C-β-, and C-γ-H), 3.59 (*s*, 3H, -$COOCH_3$), 4.90 (*t*, 1H, J = 3.5 Hz, C-α-H), 5.37 (*d* of *t*, 1H, J = 1.7 and 4.2 Hz, C-7-H), 6.22 (br. *s*, 1H, C-3-H) and no indication of any vinyl methyl group. Decoupling experiment (Figure). Downfield shift degree by Eu $(DPM)_3$[6], C-γ-H and -$COOCH_3$ > C-β-H > C-α-H > C-3-H; MS *m/e*, 274.1947 (M^+, 100%) and 201.1607 (M-CH_2COOCH_3, 72%); Existence of 3 C = C was verified from the mass spectral analysis of the reduction product (H_2/PtO_2).

Compound B. $C_{18}H_{26}O_2$; colorless oil; R_t 12.5 min[4]; UV (nm), 244[7]; IR (cm^{-1}), [film] 1744 and 1165 (saturated aliphatic ester), 1628 (C = C), 974 (*trans*-CH=CH-), and 860 (> C=CH_2); NMR (δ ppm), 1.01 (*s*, 6H) and 1.11 (*d*, J = 8.5 Hz, 3H) (3 CH_3), ≃ 1.55 (*m*, 4H, CH_2), 2.0–2.35 (*m*, 4H, C-2-, C-3-, and C-7-H), 2.95 (*m*, 2H, C-γ-H), 3.60 (*s*, 3H, -$COOCH_3$), 4.43 and 4.60 (*s* and 1H each, C-1-exoCH_2)[7], 5.48 (*m*, 2H, C-α- and C-β-H) and no indication of any vinyl methyl group. Downfield shift degree by Eu$(DPM)_3$, C-γ-H and -$COOCH_3$ > C-β-H > C-α-H; MS *m/e*, 274.1928 (M^+, 59%), 259.1677 (M-CH_3, 100%), and 201.1623 (M-CH_2COOCH_3, 25%); Existence of 3 C = C was verified from the mass spectral analysis of the reduction product (H_2/PtO_2).

Results and discussion. The spectral data for the compounds A and B are congruent with the structures (III) and (IV), respectively. Because of the low solubility of (II) in H_2SO_4, no effort has been made to obtain the direct NMR-evidence on the formation of the intermediate

[1] K. Tsukida, M. Ito and F. Ikeda, Experientia *29*, 1338 (1973).
[2] Prepared according to (a); mp. 157° (from acetone), UV 319 nm (EtOH), characterized by IR, NMR, and MS. a) I. Heilbron, E. R. H. Jones and D. G. O'Sullivan, J. chem. Soc. *1946*, 866; b) K. Eiter, E. Truscheit and H. Oediger, Angew. Chem. *72*, 948 (1960).
[3] No successful separation was effected on ordinary chromatographic columns, TLC plates, or by distillation.
[4] Pglc: 1.5% OV-17/Shimalite W, column 10′ × 3/8″, injector 215°, column 170°, detector 235°, He 150 ml/min. Glc: 1.5% OV-17/Shimalite W, 4 mm × 1 m, 170°, 140°, 200°, N_2 60 ml/min.
[5] UV-spectra were taken in ether, and NMR in CCl_4 solutions.
[6] K. Tsukida, M. Ito and F. Ikeda, Chem. Pharm. Bull. *21*, 248 (1973).
[7] T. S. Sorensen, Can. J. Chem. *42*, 2768 (1964). – M. Kuzuya and H. Hart, Tetrahedron Lett. *1973*, 4202.



(1) rearrangement (2) isomerization

cation[8]. Considering the results obtained in the previous communication[1], however, all experimental data can be explained most reasonably through a mechanism shown in the Scheme. Thus, the reaction is believed to involve the intermediacy of the cyclized, hydrindene cation (V), which would be followed competitively[9] by the rearrangement (a) or (b). In spite of many valuable reports on the conjugated polyenylic cations[10], little is known on the properties and chemical structures of the quenched products derived from the protonated, conjugated trienoic or tetraenoic acid. It has been disclosed by our studies that the conjugated trienoic acid undergoes double-cyclization in H_2SO_4 to yield almost exclusively saturated lactones, whereas the conjugated tetraenoic acid affords the cyclized and subsequently rearranged, unconjugated acid isomers almost quantitatively, both via the same type of key intermediate. Thus, our finding offers an additional contribution to the chemistry of conjugated polyenoic acids of biological interest, including vitamin A acid.

Zusammenfassung. Es wird eine neuartige, säurekatalysierte Zyklisierung und Umlagerung an einer konjugierten Tetraencarbonsäure beschrieben.

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[8] Coloration of (II) in H_2SO_4: 368 nm (80% H_2SO_4), 364 (85%), 360 and 462 (90%; two peaks turn into a single absorption band at 364 nm in a few min). These values strongly suggest a formation of certain dienylic cyclized cation as a main species. The coloration can be regenerated either from the compound A (369 nm in 80% H_2SO_4) or from B (361 nm, 80%).

[9] The competition seems to be dependent on the concentration of H_2SO_4 used. Glc analysis[4] indicates that the formation ratio of A to B is 7:2 (80% H_2SO_4), 2:1 (85%), and 1:4 (90%).

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Rabbit Kidney Alkaline Phosphatase: Role of Sialic Acid in the Heterogeneity

Heterogeneity of alkaline phosphatase has been demonstrated in a number of tissues[1–3]. The kinetic properties of the resolved activities from either liver or kidney were shown to be very nearly the same[4]. In addition, the anodic migration of fast-moving enzymes in human serum[5], liver[6] and kidney[7] were reduced by previous treatment with neuraminidase, suggesting that they were one and the same protein with differences in the carbohydrate moiety[7]. However, the sheep brain alkaline phosphatase had been resolved into 2 activities which were shown to differ in their kinetic properties[8]. It was also shown that the neuraminidase susceptible enzyme retained its original kinetic behaviour after the removal of the terminal sialic acid[9].

We wish to report that, of the 3 isoenzymes in rabbit kidney, only the slow-moving component in acrylamide gel was found to be a sialoprotein. Further, the kinetic properties remain unaffected after the treatment with neuraminidase.

Experimental. Rabbits, of both sexes aged about 45 days, were sacrificed and the kidneys (weighing 5 to 6 g) were homogenized in 0.01 *M tris*-HCl buffer, pH 7.5. Subsequently, the material was extracted with butanol[10], dialyzed and then loaded on DEAE-cellulose column, previously washed and equilibrated with 0.01 *M tris*-HCl buffer, pH 7.5, according to the method of PETERSON and SOBER[11]. Protein in the enzyme solution was estimated by the method of LOWRY et al.[12] using bovine serum albumin as standard.

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[12] O. H. LOWRY, N. J. ROSENBUROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

Table I. a) Kinetics of normal and neuraminidase treated Enz I[a]

Substrate	$K_m \times 10^{-3}$	
	Normal	Neuraminidase treated
Phenylphosphate	3.3	2.5
β-Glycerophosphate	5.0	5.5
α-Glycerophosphate	4.0	4.0
3′-AMP	6.6	6.5
5′-AMP	6.0	5.5

b) Effect of activators and inhibitors

Modifiers	Concentration (*M*)	Original activity (%)	
		Normal	Neuraminidase treated
None	–	100	100
L-Phenylalanine	6.6×10^{-3}	110	110
Phosphate	6.6×10^{-3}	80	85
Mg^{++}	3.3×10^{-3}	300	300
Zn^{++}	6.6×10^{-4}	0	0
Be^{++}	6.6×10^{-4}	0	0