The CMR characteristics of jungermanool

Carbon	Chemical shift	Multiplicity
1	31.9[a]	t
2	18.8	t
3	32.7[a]	t
4	38.6	s
5	48.6	d
6	27.5	t
7	36.2	t
8	148.2	s
9	77.3	s
10	43.6	s
11	28.9	t
12	41.2	t
13	73.4	s
14	145.4	d
15	111.4	t
16	28.0	q
17	106.5	t
18	24.4	q
19	17.8	q
20	17.8	q

[a] May be interchanged.

[7] A. T. BLOMQUIST and D. T. LONGONE, J. Am. chem. Soc. *79*, 3916 (1957). W. J. BAILEY, R. L. HUDSON and C.-W. LIAO, J. Am. chem. Soc. *80*, 4358 (1958). D. H. R. BARTON and G. J. GUPTA, J. chem. Soc. *1962*, 1961.

[8] The ^{13}C-NMR-spectrum was obtained in $CDCl_3$ solution on JEOL JNM-FX60 FT spectrometer at the condition of pulse repetition 2 sec, accumulation 300 times and frequency range 4 KHz.

[9] S.-O. ALMQVIST, C. R. ENZELL and F. W. WEHRLI, Acta chem. scand. *B 29*, 695 (1975). B. L. BUCKWALTER, I. R. BURFITT, A. A. NAGEL, E. WENKERT and F. NÄF, Helv. chim. Acta *58*, 1567 (1975).

ν 3640, 3550, 1715 cm^{-1}; δ 0.87, 0.93, 1.01, 2.02, each 3H, s; δ 4.43, 4.78, each 1H, s, due to loss of the vinyl group was obtained as well as in the case of manool (II→VI)[6]. However, compound V was characterized as a keto-alcohol with a tertiary hydroxyl group, while the oxidation product from manool was a ketone (VI), $C_{18}H_{30}O$ (M^+ 262); $[\alpha_D]$ −31.4°; ν 1715 cm^{-1}. These facts revealed jungermanool as a derivative of manool with an additional tertiary hydroxyl group. To determine the position of the remaining hydroxyl group, the keto-alcohol was further treated with $SOCl_2$ in pyridine to give a diene (VII), $C_{18}H_{28}O$ (M^+ 260); $[\alpha]_D$ +41.3°; ν 1715, 1644, 880 cm^{-1}; δ 0.90, 1.06, 1.06, 2,03, each 3H, s; δ 2.72, 2H, d, J=3.8; δ 4.55, 4.88, each 1H, br.s; δ 5.41, 1H, t, J= 3.8, which showed a UV-absorption band, $\lambda_{max}^{isooct.}$ 213 nm (ε 7150), attributable to a conjugated diene system, bisexocyclic to a cyclohexane ring[7]. The formation of this conjugated diene system is reasonably explained by locating the additional tertiary hydroxyl group on C-9 of the labdane carbon skeleton. The gross structure of jungermanool is thus determined to be labda-8 (17), 14-dien-9, 13-diol (I).

This structure was supported by the fact that in measuring PMR-spectra of jungermanool and manool with application of the shift reagent, Eu(dpm)$_3$, the exomethylene protons of jungermanool, which are close to the C-9 hydroxyl group in the proposed structure, showed the most remarkable variation between the signal shifts of the corresponding protons of both alcohols. A further support was also obtained by the single frequency offset resonance ^{13}C-NMR-spectrum (Table)[8,9] which consists of 5 singlets (1 olefinic carbon: 148.2; 2 carbinyl carbons: 73.4, 77.3; 2 fully substituted carbons: 38.6, 43.6); 2 doublets (1 olefinic: 145.4, 1 methine carbon: 48.6); 9 triplets (2 olefinic: 106.5, 111.4; 7 usual methylenes: 18.8, 27.5, 28.9, 31.9, 32.7, 36.2, 41.2) and 4 methyl quartets (17.8, 17.8, 24.4, 28.0).

Synthetic Simulation of Nonribosomal Peptide Biosynthesis. A Dual Role of Alkylthiol Esters in Peptide Synthesis

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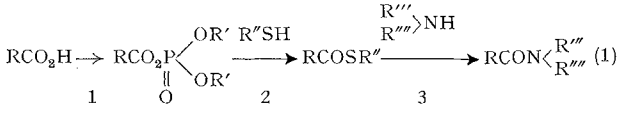
Summary. Coupling of peptide alkylthiol esters with amino acid derivatives in the presence of pivalic acid or 2-hydroxypyridine proceeds without racemization. A dual role of alkylthiol esters as protective and reactive functions in peptide synthesis was well proved.

Recent studies on nonribosomal peptide biosynthesis[1], represented by gramicidin S and tyrocidine biosynthesis, have revealed that α-amino acids are activated with ATP to give mixed anhydrides (step 1) which are transformed into alkylthiol esters (step 2), followed by the peptide bond formation (step 3):



(1)

We have already reported[2] a general method for preparing thiol esters from carboxylic acids and thiols using diethyl phosphorocyanidate[3] ($NCPO(OEt)_2$, DEPC) or diphenyl phosphorazidate[4] ($N_3PO(OPh)_2$, DPPA) in combination with triethylamine[5]. This thiol ester formation reaction will be regarded as steps 1 and 2 in equation 1 because the intermediates will possibly be mixed phosphoric carboxylic anhydrides[4]. We now wish to report realization of the laboratory analogy for step 3

[1] F. LIPMANN, Acc. chem. Res. *6*, 361 (1973), and references therein.

[2] S. YAMADA, Y. YOKOYAMA and T. SHIOIRI, J. org. Chem. *39*, 3302 (1974).

[3] S. YAMADA, Y. KASAI and T. SHIOIRI, Tetrahedron Lett. *1973*, 1595.

[4] T. SHIOIRI, K. NINOMIYA and S. YAMADA, J. Am. chem. Soc. *94*, 6203 (1972). – T. SHIOIRI and S. YAMADA, Chem. Pharm. Bull. *22*, 849, 855, 859 (1974).

[5] S. YAMADA, N. IKOTA, T. SHIOIRI and S. TACHIBANA, J. Am. chem. Soc. *97*, 7174 (1975).

in nonribosomal peptide biosynthesis[6], promizing practical value of alkylthiol esters in peptide synthesis[7].

In order to detect the possible occurrence of racemization during coupling of alkylthiol esters with amino functions, we investigated the supersensitive YOUNG test[8] involving the synthesis of Bz-Leu-Gly-OEt[9] from Bz-L-Leu-SEt and H-Gly-OEt. Little, if any, racemization was observed during the coupling. The reaction proceeds smoothly when pivalic acid or 2-hydroxypyridine is present as a bifunctional catalyst[10] resembling nonribosomal peptide biosynthesis. Preferred solvents are pyridine, dimethylformamide, and benzene. A typical procedure is as follows. A mixture of Bz-L-Leu-SEt[2] (0.279 g, 1 m*M*), H-Gly-OEt·HCl (0.167 g, 1.2 m*M*), pivalic acid (0.10 g, 1 m*M*), and triethylamine (0.17 g, 1.7 m*M*) in pyridine (2 ml) was stirred at 20° for 21 h. Benzene-ethyl acetate (1:1, 50 ml) was added to the reaction mixture, which was worked up with aqueous acid (5% HCl or 5% citric acid) and saturated aqueous $NaHCO_3$ and dried. The evaporated residue was purified by a silica gel column chromatography with *n*-hexane and ethyl acetate (9:1→1:1) to give Bz-Leu-Gly-OEt (0.249 g, 89%), mp 158–162°, $[\alpha]_D^{20}$–32.7° (c = 2.9, EtOH), corresponding to 96% L-isomer[11]. When no bifunctional catalyst was present under similar reaction conditions, Bz-Leu-Gly-OEt was obtained in poor yield with the recovery of most of the starting Bz-L-Leu-SEt. Furthermore none of Bz-Leu-Gly-OEt was produced when Bz-L-Leu-OMe was used in place of Bz-L-Leu-SEt, proving the functional specificity in the method.

A dual role of alkylthiol esters as protective and reactive functions[12] was examined on the IZUMIYA racemization test[13] which involves coupling of Boc-Gly-L-Ala-SEt with H-L-Leu-OBu^t. Z-L-Ala-OH was condensed with ethanethiol using DEPC[2] in the presence of triethylamine in dimethylformamide to give Z-L-Ala-SEt, mp 42–43°, $[\alpha]_D^{20}$–12.2° (c = 2.0, $CHCl_3$), in 94% yield. Deblocking with hydrogen bromide in acetic acid afforded H-L-Ala-SEt·HBr in 96% yield, which was coupled with Boc-Gly-OH by means of DPPA and triethylamine in dimethylformamide[4,5] to yield Boc-Gly-L-Ala-SEt, mp 76–79°, $[\alpha_D^{20}]$–64° (c=2.2, EtOH), in 82% yield. Condensation of the thiol ester with H-L-Leu-OBu^t (2 equiv) in the presence of pivalic acid in pyridine or dimethylformamide at 20° for 21 h as in the YOUNG test gave Boc-Gly-L-Ala-L-Leu-OBu^t in 90% yield, which was treated with trifluoroacetic acid to furnish H-Gly-L-Ala-L-Leu-OH. No peak of H-Gly-D-Ala-L-Leu-OH was detected by the amino acid analyzer[13], revealing that the coupling of the peptide alkylthiol ester with the amino acid ester in the presence of the bifunctional catalyst proceeds without any racemization.

Additional evidence of utility of alkylthiol esters was obtained by the preparation of Boc-L-Trp-L-Leu-L-Asp-L-Phe-NH_2 bearing the same gastric acid secretory activity as that of diagnostically useful tetragastrin Boc-L-Trp-L-Met-L-Asp-L-Phe-NH_2[14]. Boc-L-Asp(OBzl)-SEt, quantitatively obtained from Boc-L-Asp(OBzl)-OH and ethanethiol using either DEPC or DPPA method[2], was deblocked with 2.3 *N* HCl-ethyl acetate to give H-L-Asp(OBzl)-SEt·HCl. Stepwise addition of Boc-L-Leu-OH·H_2O and Boc-L-Trp-OH using DEPC[3,5] as a coupling reagent and 2.3 *N* HCl-ethyl acetate as a deprotecting reagent afforded Boc-L-Trp-L-Leu-L-Asp(OBzl)-SEt, mp 117–119°, $[\alpha]_D^{20}$–48.9° (c=0.97, EtOH), in 46% yield from Boc-L-Asp(OBzl)-SEt. Coupling of the tripeptide derivative with H-L-Phe-NH_2 in dimethylformamide in the presence of pivalic acid as a catalyst gave Boc-L-Trp-L-Leu-L-Asp(OBzl)-L-Phe-NH_2 in 47% yield. Hydrogenolysis over 5% Pd-C afforded Boc-L-Trp-L-Leu-L-Asp-L-Phe-NH_2[15], mp 215–219° (dec), $[\alpha]_D^{20}$–42° (c= 0.18, DMF). Inertness of the β-benzyl ester group of Asp toward the coupling reaction exhibits the functional specifity of the process.

The synthetic simulation of nonribosomal peptide biosynthesis thus highlights a dual role of alkylthiol esters as both protective and reactive functions in peptide synthesis, which may promise the added variation in laboratory strategy for the peptide synthesis.

[6] Biosynthesis of hippuric acid also proceeds according to eq. 1; E. E. CONN and P. K. STUMPF, *Outlines of Biochemistry* (John Wiley and Sons, Inc., New York, N.Y. 1972), chapter 19.

[7] On previous attempts concerning aminolysis of thiol esters, see T. C. BRUICE, *Organic Sulfur Compounds* (Ed. N. KHARASCH; Pergamon Press, New York and London 1961), vol. 1, chapter 35. – T. C. BRUICE and S. J. BENKOVIC, *Bioorganic Mechanism* (W. A. Benjamin, New York and Amsterdam 1966), vol. 1, chapter 3.

[8] M. W. WILLIAMS and G. T. YOUNG, J. Chem. Soc. *1963*, 881.

[9] Symbols and abbreviations are in accordance with rules approved by IUPAC-IUB commission on biochemical nomenclature, Pure appl. Chem. *40*, 315 (1974).

[10] N. NAKAMIZO, Bull. Chem. Soc. Japan *42*, 1071, 1078 (1969), and references therein.

[11] Lit.[8] mp 156.5–157°, $[\alpha]_D^{20}$ – 34° (c = 3.1, EtOH).

[12] Recently a dual role of tert-butylthiol ester as protective and reactive functions was reported in the lactone formation of the macrolide antibiotics; S. MASAMUNE, H. YAMAMOTO, S. KAMATA and A. FUKUZAWA, J. Am. chem. Soc. *97*, 3513 (1975); S. MASAMUNE, S. KAMATA and W. SCHILLING, J. Am. chem. Soc. *97*, 3515 (1975).

[13] N. IZUMIYA, M. MURAOKA and H. AOYAGI, Bull. chem. Soc. Japan, *44*, 3391 (1971).

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[15] G. W. KENNER, J. J. MENDIVE and R. C. SHEPPARD, J. chem. Soc. (C) *1968*, 761; mp 217°, $[\alpha]_D^{22}$ – 46.5° (c = 0.35, DMF).

The Structure of Durantoside IV Tetraacetate

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Summary. Durantoside IV tetraacetate was isolated from *Duranta repens* Linn and identified by physical and chemical evidence.

Material and methods. We have isolated several bitter principles from the ethanol extractive of the leaves of *Duranta repens* Linn, and one component, durantoside IV tetraacetate, was purified by acetylation.

Results and discussion. Durantoside IV tetraacetate 1 forms colorless needles, m.p. 215–217°, $(\alpha)_D$ –47.9° $C_{36}H_{42}O_{19}$, λ_{max} 220.5, 225 and 284 nm. The UV-spectrum (λ_{max} 225 nm) and the NMR-spectrum (τ_{CDCl_3} 2.71, s,