NEW ANTIBIOTICS, METHYLENOMYCINS A AND B

III. CHEMICAL MODIFICATIONS OF METHYLENOMYCIN A AND STRUCTURE-ACTIVITY CORRELATIONS IN METHYLENOMYCINS

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(Received for publication February 28, 1974)

Chemical modification of methylenomycin A afforded broadened spectrum as well as stronger antibiotic activity. In addition to the original antibacterial activity of methylenomycin A, the alkyl ester derivatives of the antibiotic possessed antifungal activity. With regard to structure-activity relationship, the existence of the α, β-unsaturated carbonyl function, epoxide and cyclopentane ring was found to be essential for antibacterial activity, and the alkyl ester, C_3-carbonyl group, epoxide and cyclopentane ring for the antifungal activity.

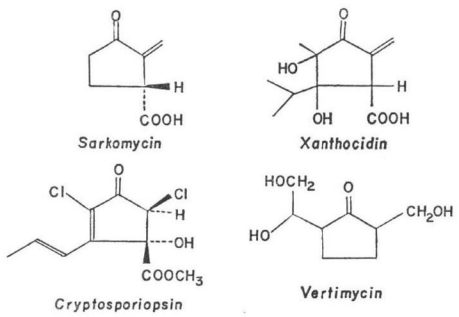
Methylenomycins A and B consist of a compact molecule fused with several functions which are assumed to be related to the biological activity of these antibiotics.[1,2] As reported in the previous paper,[2] methylenomycin A is composed of three functions: a ketone group conjugated to a terminal methylene, a carboxylic acid and an epoxide group attached to the cyclopentane ring. The present paper deals with chemical modification of methylenomycin A aiming to obtain stronger antibiotic activity as well as to define the basic structural requirements for methylenomycin activity.

Several antibiotics with cyclopentane nuclei have been reported as follows:

Sarkomycin[3~8] ($C_6H_8O_3$, $[\alpha]_D^{15}-32.5°$) is closely related to methylenomycins. It was isolated from the cultured broth of *Streptomyces erythrochromogenes* by UMEZAWA *et al.* It has antitumor as well as antibacterial activity, while antitumor activity in mice is not demonstrable with the methylenomycins. The hydrogenation product of sarkomycin is as active as sarkomycin itself against EHRLICH ascites carcinoma cells, but is inactive against *Staphylococcus aureus* FDA 209P.

Cryptosporiopsin[9~11] ($C_9H_{10}Cl_2O_4$, $[\alpha]_D^{20}+61.5°$) produced by *Sporornia affinis*, *Cryptosporiosis* sp., and *Batula alleghaniensis* was isolated by MCGAHREN *et al.* This antibiotic, containing chlorine in its molecule, is distinguished from other cyclopentane antibiotics with respect to its antifungal activity along with its activity against gram-positive bacteria.

Chart 1.



Xanthocidin[12,12] ($C_{11}H_{16}O_5$, $[\alpha]_D^{25}+16.7$), isolated from the culture filtrate of *Streptomyces* sp. by ASAHI *et al.*, is also closely related to the methylenomycins in its chemical structure. It has specific activity against *Xanthomonas oryzae* and *Escherichia coli*. Another cyclopentane antibiotic without α, β-unsaturated

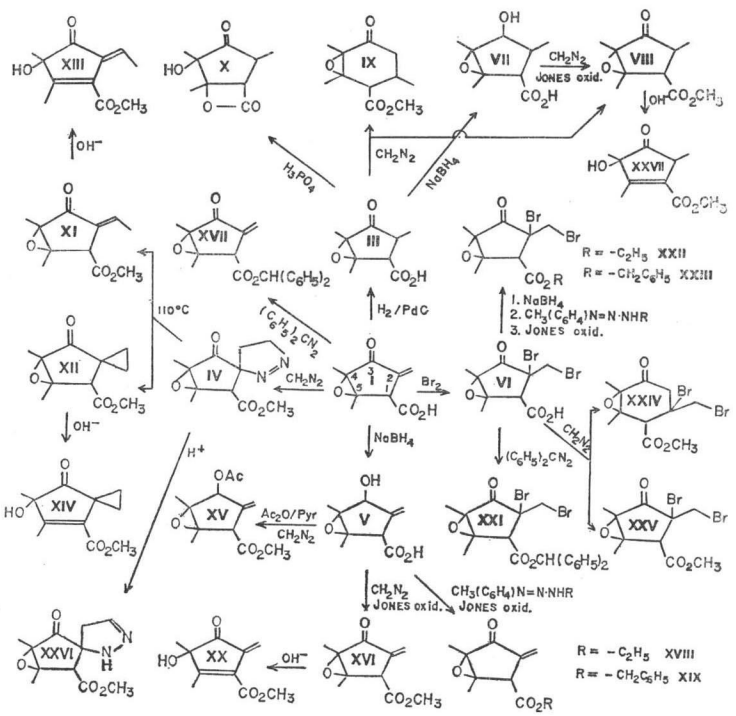
carbonyl group such as vertimycin[14] has also been reported. Vertimycin was isolated from the culture filtrate of *Streptomyces* sp. JA 4498 by STRAUS, and is active against gram-positive bacteria, some species of *Nocardia* and tumors.

Syntheses and Antimicrobial Activities of Methylenomycin A Derivatives

Hydrogenation of methylenomycin A (**I**) on palladium-carbon yielded its C_2-methyl derivative (**III**) with complete loss of antimicrobial activity. Bromination of compound **I** afforded dibromo-derivative (**VI**) with stronger antimicrobial activity against gram-positive and gram-negative bacteria than that of compound **I**. Migration of the exoethylenic double bond into the cyclopentane ring, as generally expected, was unsuccessful. The reaction of compound **I** with diazomethane provided a methyl ester of 2, 3-pyrazoline derivative (**IV**). In the case of the 2, 3-pyrazoline derivative of methylenomycin A, two compounds, **XI** and **XII**, were derived with the evolution of nitrogen on heating in toluene at 120°C for 30 minutes. The former was a C_2-ethylidene methyl ester (homomethylenomycin methyl ester) and the latter was the C_2-cyclopropane methyl ester. Compound **XI** was converted quantitatively to C_2-1, 2-pyrazoline derivative (**XXVI**) in the presence of a catalytic amount of hydrobromic acid. But compounds **XI** and **XII** could not be obtained from compound **XXVI** by the same procedure described for 2, 3-pyrazoline derivative. All of the derivatives modified at the C_2 position, such as 2-methyl derivative (**III**), 2-cyclopropane derivative (**XII**) and 2-pyrazoline derivatives (**IV** and **XXVI**), except 2-ethylidene derivative (**XI**), showed a complete loss of antibacterial activity.

For the modification of the C_3 carbonyl group, compounds **I** and **III** were reduced with $NaBH_4$ to yield inactive C_3-hydroxy derivatives (**V** and **VII**). Furthermore, the 3-O-acetyl derivative (**XV**) also lacked antimicrobial activity.

Chart 2.



The epoxide of methylenomycin A was comparatively stable and resisted cleavage by ordinarily used nucleophiles or hydrogenation over palladium carbon. This epoxide was disrupted by 85% phosphoric acid or 1 N NaOH treatment in alcohol. Cleavage of the epoxide was achieved by treatment of compound **III** in phosphoric acid to yield inactive crystalline β-lactone monohydroxy derivative (**X**). On the other hand, methyl ester derivatives, **XVI, XI** and **XII,** were also treated in 1 N NaOH in alcohol at 50°C for 30 minutes to form the corresponding 1, 5-dehydro-4-monohydroxyl derivatives, **XX, XIII** and **XIV,** respectively. All these epoxide-disrupted monohydroxyl derivatives lacked antibiotic activity.

A conversion of the cyclopentanone ring to a cyclohexanone ring was achieved in low yield by condensation of methylenomycin A derivatives with diazomethane. Compounds **III** and **VI** were converted to compound **IX** and **XXIV**, respectively, with loss of activity.

Methyl ester derivatives, **XVI** and **XXV**, were prepared by reaction with diazomethane. As described above not only stronger antibacterial activity than methylenomycin A but also antifungal activity was found in these methyl ester derivatives.

From the preliminary considerations on the biological activities of methylenomycins A (**I**) and B (**II**), it is evident that the carboxyl group is not essential for the activity because methylenomycin B (**II**), a decarboxylated derivative of methylenomycin A (**I**), showed similar antibacterial activity to that of methylenomycin A (**I**). Antifungal activity of methyl ester derivatives of methylenomycin A (**I**), however, provided evidence that the presence of an ester group was necessary for antifungal activity.

The information on the biological activities of the methyl ester derivatives suggested modification of the ester group by elongation of its carbon chain or displacement of the aliphatic acyl group by aromatic group might provide a practical approach for increasing activity.

The ethyl ester (**XVIII**) and benzyl ester (**XIX**) were prepared from compound **V** by the reaction with the corresponding l-methyl-3-*p*-tolyl-triazene derivatives followed by JONES oxidation. Diphenyl ester (**XVII**) was obtained directly from methylenomycin A (**I**) by treatment with diphenyl diazomethane.

The ethyl (**XXII**), benzyl (**XXIII**) and diphenyl (**XXI**) esters of dibromo-derivatives were prepared by the same procedures described above. The antimicrobial activities against *Proteus vulgaris* and *Trichophyton interdigitale* of methyl ester derivatives (**XVI, XXV**) were four times or more than those of other ester derivatives, although the activity against *Staphylococcus aureus* was not affected by changing acyl group.

Structure-Activity Correlations

Taking these results into consideration, it is obvious that the carbonyl group at C_3 and the terminal methylene at C_2 are essential for the activity of methylenomycin A (**I**), but that an ethylidene group can replace for the terminal methylene.

Therefore, it was concluded that the existence of an α, β-unsaturated carbonyl function was most important for the antibacterial activity of methylenomycins and that epoxide and cyclopentane ring were also essential for the activity.

All of the compounds with antifungal activity retained C_1 carboxyl ester group. Among them, the methyl ester derivative was found to be most active, as shown in Table 1.

Table 1. Antimicrobial activities (MIC) of methylenomycin derivatives

Compound	MIC (mcg/ml)			Compound	MIC (mcg/ml)		
	Staphylococcus aureus 209P JC-2	*Proteus vulgaris* OX-19	*Trichophyton interdigitale*		*Staphylococcus aureus* 209P JC-2	*Proteus vulgaris* OX-19	*Trichophyton interdigitale*
I	200	25	>400	**XV**	>400	>400	>400
II	100	50	>400	**XVI**	12.5	6.3	6.3
III	>400	>400	>400	**XVII**	6.3	50	>400
IV	>400	>400	100	**XVIII**	12.5	25	25
V	>400	>400	>400	**XIX**	12.5	50	>400
VI	50	3.1	>400	**XX**	>400	>400	>400
VII	>400	>400	>400	**XXI**	6.3	25	25
VIII	>400	>400	>400	**XXII**	12.5	25	12.5
IX	>400	>400	>400	**XXIII**	6.3	25	25
X	>400	>400	>400	**XXIV**	>400	>400	400
XI	200	50	50	**XXV**	6.3	3.1	6.3
XII	400	200	100	**XXVI**	>400	>400	200
XIII	400	>400	>400	**XXVII**	>400	>400	>400

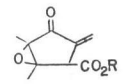
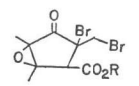
Some differences in structure-activity relationship were observed between antibacterial and antifungal activities of methylenomycin A derivatives. The antibacterial and antifungal activities of the ethyl ester derivatives were remarkably depressed comparing with those of methyl esters. The antifungal activity significantly decreased with an increase in the length of the carbon chain of aliphatic groups. Aromatic esters were all inactive except for dibromo-methylenomycin A diphenyl ester (**XXI**). These facts clearly indicated that for antifungal activity useful acyl groups were restricted to aliphatic types, and especially to methyl ester of methylenomycin A (**I**). The α, β-unsaturated carbonyl group was not critical for antifungal activity because C_2-cyclopropane methyl ester derivative (**VII**) possessed weak antifungal activity. The ketone group at C_3 position was a basic requirement for the antifungal activity since the C_3-hydroxyl methyl ester derivatives were all inactive against fungi. The epoxide and cyclopentane ring were also assumed to be essential factors for the antifungal activity because cyclohexane and epoxide-disrupted derivatives were all inactive.

Table 2 compares the biological activities of selected derivatives of methylenomycin A (**I**) and dibromo-methylenomycin A (**VI**). Methylenomycin A or dibrmo-methylenomycin A was active against gram-positive and gram-negative bacteria, especially active against *Proteus*, but inactive against fungi. On the other hand, the methyl ester derivatives of these two compounds mentioned above indicated broadened and increased antibiotic activity. They were active against some species of fungi as well as gram-positive and gram-negative bacteria.

The biological activities of their ethyl and benzyl esters were weaker than their parent compounds but had a similar tendency to their corresponding methyl and diphenyl ester derivatives. In the cases of diphenyl ester derivatives, antibacterial activity increased against gram-positive bacteria, but decreased against gram-negative bacteria and antifungal activity was completely lost.

The toxicities (LD_{50}) of these derivatives in mice were examined by intraperitoneal injection as shown in Table 2. The methyl ester of methylenomycin A was only slightly toxic and had

Table 2. Comparison of MIC and toxicity of selected derivatives of methylenomycin A and dibromo-methylenomycin A.

Test organism	MIC (mcg/ml)					
						
	-H	$-CH_3$	$-CH(C_6H_5)_2$	-H	$-CH_3$	$-CH(C_6H_5)_2$
Staphylococcus aureus 209P JC-2	200	12.5	6.3	50	6.3	6.3
S. aureus 56	400	50	25	100	25	100
Sarcina lutea PCI 1001	25	25	6.3	25	6.3	6.3
Bacillus subtilis PCI 219	25	25	12.5	50	6.3	200
Alcaligenes faecalis	100	50	6.3	25	6.3	12.5
Escherichia coli NIHJ JC-2	200	50	>400	100	12.5	>400
E. coli K-12	400	200	>400	>400	50	>400
E. coli CM, TCf	25	12.5	50	25	3.1	400
Proteus vulgaris OX-19	25	6.3	50	25	3.1	25
P. mirabilis	200	50	>400	100	25	>400
P. rettgeri	50	12.5	50	12.5	6.3	100
Klebsiella pneumoniae PCI 602	200	50	25	200	6.3	100
Pseudomonas aeruginosa	50	50	50	200	25	50
Mycobacterium smegmatis ATCC 607	>400	>400	>400	>400	>400	>400
Aspergillus oryzae	>400	400	>400	>400	400	25
Penicillium chrysogenum	>400	400	>400	>400	400	>400
Candida albicans	>400	100	>400	>400	50	>400
Trichophyton mentagrophytes	>400	12.5	>400	>400	25	50
T. interdigitale	>400	6.3	>400	>400	6.3	25
T. rubrum	>400	3.1	>400	>400	3.1	12.5
Epidermophyton floccosum	>400	3.1	>400	>400	6.3	12.5
Blastomyces brasiliensis	>400	3.1	>400	>400	6.3	25
Piricularia oryzae	>400	50	>400	>400	50	200
Toxicity in mice (ip) LD_{50} (mg/kg)	75	250	>400	175	<25	>200

stronger antibiotic activity. The methyl ester of dibromomethylenomycin A (**VI**) showed the strongest antibiotic activity, but also the highest toxicity amomg all the biologically-active derivatives. The low toxicity of the diphenyl esters was assumed to depend on their solubilities because they were almost insoluble in many organic solvents or water.

Experimental

The preparative procedures on some derivatives have been described in the previous paper.[2] They are as follows: Compounds **III**, **IV**, **V**, **VI**, **VII**, **VIII**, **X**, **XI**, **XII** and **XXVII**.

Compound **XIII**. Compound **XI** (100 mg) was dissolved in 5 ml of 1 N NaOH in 50% aqueous MeOH and allowed to stand for 30 minutes at room temperature. After evaporation of the reaction mixture under reduced pressure, the concentrate was extracted with ether and purified by preparative thin-layer chromatography using silica gel developed with benzene and eluted with EtOAc (73 mg); colorless oil, IR $\nu_{max}^{CHCl_3}$ 1730, 1710, 1650 cm^{-1}. Found: C, 63.20; H, 6.60%. Calcd. for $C_{11}H_{14}O_4$: C, 62.86; H, 6.67%.

Compound **XIV**. Compound **XII** (75 mg) was treated with 5 ml of 1 N NaOH in aqueous MeOH and reacted for 30 minutes at room temperature. The reaction mixture was evaporated *in vacuo* to remove MeOH, neutralized with 1 N HCl and extracted with EtOAc. The extract was purified on a silica gel column packed and eluted with benzene. Compound **XIV** was obtained as colorless oil (50 mg); IR $\nu_{max.}^{KBr}$ 1730, 1705, 1625 and 1225 cm^{-1}; NMR $\delta_{ppm}^{CDCl_3}$ 1.60 (s, 3H, =C($\underline{CH_3}$)–), 2.23(s, 3H, HO–C($\underline{CH_3}$)–), 3.76(s, 3H, –COO$\underline{CH_3}$), two methylenes at 1.27 and 1.60(m, 4H, J=10 and 4.0 Hz). Found: C, 62.30; H, 6.52 %. Cacld. for $C_{11}H_{14}O_4$: C. 62.86; H, 6.67 %.

Compound **XV**. Compound **V** (300 mg) was dissolved in 1 ml of pyridine, 3 ml of acetic anhydride was added, and the mixture was allowed to stand for 15 hours at room temperature. The reaction mixture was poured on ice, and the resulting mixture extracted with EtOAc at pH 3.0. The extract was evaporated to dryness at reduced pressure. The monoacetyl derivative thus obtained was immediately reacted with diazomethane and yielded methyl ester derivative **XV**.

Compound **XVI**. Compound **V** (978 mg), in $CHCl_3$, was treated with diazomethane. The reaction mixture was evaporated *in vacuo*. The oily mass thus obtained was purified by column chromatography on a silica gel packed with benzene. Elution was carried out with benzene-EtOAc (95:5) and 650 mg of the methyl ester of compound **V** was obtained. To the acetone solution (10 ml) of the methyl ester of compound **V** (600 mg), was added 1 ml of JONES reagent and the mixture was stirred for 60 minutes at room temperature. After addition of 30 ml of EtOAc to the reaction mixture, the upper layer was washed three times with 10-ml portions of water saturated with sodium chloride followed by evaporation to dryness *in vacuo* and purified on a silica gel column packed with benzene and eluted with benzene-EtOAc (97:3). Compound **XVI** was obtained as 450 mg of a colorless oil: $C_{10}H_{12}O_4$, *m/e* 196, IR $\nu_{max}^{CHCl_3}$ 1740, 1645 cm^{-1}.

Compound **XVII**. To compound **I** dissolved in 5 ml of EtOAc (310 mg) was added freshly prepared diphenyl diazomethane until the end of bubbling. The reaction mixture was evaporated *in vacuo* and the crude crystalline material thus obtained was treated with *n*-hexane to afford almost pure product (565 mg): IR $\nu_{max}^{CHCl_3}$ 1740, 1645 cm^{-1}; NMR; $\delta_{ppm}^{CDCl_3}$ 3.76 (m, 1H, –$\underline{CH}$–$CO_2CH(C_6H_5)_2$, J=2.0, 2.0 Hz), 6.14(d, 1H, $\frac{H}{H}$>C=, J=2.0 Hz), 5.32 (d, 1H, $\frac{H}{\underline{H}}$>C=, J=2.0 Hz), 6.92 (s, 1H, –C$\underline{H}(C_6H_5)_2$), 7.20 (10H, –CH($\underline{C_6H_5}$)$_2$. Found: C 75.86; H, 5.74 %. Calcd. for $C_{22}H_{20}O_4$: C, 75.85; H, 5.72 %.

Compound **XVIII**. To compound **V** (1 g) dissolved in 15 ml of ether was added an ether solution of 1-ethyl-3-*p*-tolyltriazene (1.3 g) and the mixture was allowed to stand for 60 minutes at room temperature. The reaction mixture was washed twice with 10 ml of 0.5 N HCl, and then with water saturated with sodium chloride and evaporated *in vacuo*. The resulting oily product, dissolved in 20 ml of acetone, was oxidized with 2 ml of JONES reagent at room temperature. The reaction mixture was washed with water saturated with sodium chloride. After evaporation of the acetone, it was applied to a silica gel column packed with benzene and eluted with the same solvent, and then 134 mg of purified compound **XVIII** was obtained as slightly yellowish oil: IR $\nu_{max}^{CHCl_3}$ 1735, 1640 cm^{-1}; NMR $\delta_{ppm}^{CDCl_3}$ 1.20 (t, 3H, –COOCH$_2$C$\underline{H_3}$, J=2.5 Hz), 4.14 (q, 2H, –COO$\underline{CH_2}$CH$_3$, J=2.5 Hz).

Compound **XIX**. Compound **V** (500 mg) in 10 ml of ether was added to 1-benzyl-1-3-*p*-tolyltriazene (1.0 g) at room temperature and allowed to stand for 30 minutes. The reaction mixture was washed with 10 ml of 1 N HCl, then washed with water to remove excess of hydrochloric acid. The ether layer was concentrated *in vacuo* (730 mg). The resulting crude benzyl ester of compound **V** (700 mg), in 10 ml of acetone, was oxidized with 2 ml of JONES reagent at 0°C and allowed to stand for 60 minutes at room temperature. After addition of 30 ml of EtOAc to the reaction mixture, the upper layer was collected and washed three times with water saturated with sodium chloride and evaporated to dryness. The resulting crude

compound **XIX** was purified on a silica gel column packed and eluted with benzene-EtOAc (95:5). Finally compound **XIX** was obtained as 53 mg of colorless oil: NMR $\delta_{ppm}^{CDCl_3}$ 5.15 (s, 2H, $-\underline{CH_2}-C_6H_5$), 7.33 (s, 5H, $-\underline{C_6H_5}$). Found: C, 70.50; H, 5.93%. Calcd. for $C_{16}H_{16}O_4$: C, 70.59; H, 5.88%.

Compound **XX**. Compound **XVI** (300 mg), dissolved in 5 ml of MeOH, was mixed with 5 ml of 1 N NaOH and allowed to stand for 20 hours at 37°C. After neutralization with 1 N HCl, the reaction mixture was evaporated to remove MeOH. The product was extracted with ether and purified on a silica gel column packed and eluted with benzene. Compound **XX** was obtained as a colorless oil (165 mg): *m/e* 196; IR $\nu_{max.}^{Liq.}$ 1745, 1720, 1650 cm^{-1}; NMR $\delta_{ppm}^{CDCl_3}$ 3.60 (s 3H, $-COO\underline{CH_3}$), 5.50 (s, 1H, $\underline{H}/H>C=$), 6.20 (s, 1H, $\underline{H}/H>C=$). Found: C, 61.51; H, 7.05%. Calcd. for $C_{10}H_{12}O_4$: C, 61.22; H, 7.14%.

Compound **XXI**. To compound VI in 5 ml of $CHCl_3$, diphenyl diazomethane was added and allowed to stand for one hour at room temperature. The reaction mixture was evaporated to dryness. Crude compound **XXI** extracted from the residue with *n*-hexane was purified on a silica gel column. It was eluted with *n*-hexane-benzene (4:1) and then purified compound **XXI** was obtained as a colorless oil (73 mg): Found: C, 52.15; H, 4.01; Br, 31.75%. Calcd. for $C_{22}H_{20}Br_2O_4$: C, 51.97; H, 3.94; 31.49%.

Compound **XXII**. To compound **VI** (171 mg) dissolved in 10 ml of ether was added 100 mg of 1-ethyl-3-*p*-tolyltriazene in 2 ml of ether in an ice bath and this was stirred for 2 hours at room temperature. The reaction mixture was washed three times with 1 N HCl, subsequently washed with water and concentrated *in vacuo*. The residue was applied to a silica gel column and eluted with benzene (65 mg). Found: C, 41.98; H, 4.47; Br, 32.75%. Calcd. for $C_{11}H_{14}O_4Br_2$: C, 42.42; H, 4.50; Br, 32.51%.

Compound **XXIII**. Compound **VI** (300 mg) dissolved in 10 ml of ether was reacted with 600 mg of 1-benzyl-1-3-*p*-tolyltriazene at room temperature. The reaction mixture was washed three times with 5-ml portions of 1 N HCl, and water saturated with sodium chloride and evaporated to dryness *in vacuo*. The resulting residue was purified by preparative silica gel thin-layer, developed with a mixture of benzene-EtOAc (4:1) and eluted with acetone. Compound **XXIII** was obtained as slightly yellowish oil (78 mg): Found: C, 53.55; H, 4.70; Br, 28.51%. Calcd. for $C_{16}H_{16}Br_2O_4$: C, 53.94; H, 4.74; Br, 28.24%.

Compounds **XXIV and XXV**. One gram of compound **VI** in $CHCl_3$ was treated with diazomethane. The reaction mixture was purified by silica gel column chromatography developed with a mixture of benzene-*n*-hexane (1:1). Compound **XXV** was obtained as a major component and compound **XXIV** as a minor component. Compound **XXV**. (620 mg): IR ν_{max}^{KBr} 1765, 1740 cm^{-1}; NMR $\delta_{ppm}^{CDCl_3}$ 3.84 (s, 1H, $-\underline{CH}-COOCH_3$), 3.89 (s, 3H, $-COO\underline{CH_3}$) 3.95 (ABq, 2H, $-\underline{C}(Br)-\underline{CH_2}Br$, J=12.0 Hz). Found: C, 40.75; H, 4.11; Br, 33.76%. Calcd. for $C_{10}H_{12}O_4Br_2$: C, 40.30; H, 4.03; Br, 34.00%.

Compound **XXVI**. To compound **IV** (200 mg) in 10 ml of $CHCl_3$ were added a few drops of HBr under vigorous stirring for one hour at room temperature. After washing the reaction mixture with water, it was concentrated and applied to a silica gel preparative thin-layer which was developed with a mixture of benzene-EtOAc (95:5) and eluted with acetone. Compound **XXVI** was obtained as a colorless oil (167 mg): *m/e* 238; IR $\nu_{max}^{CCl_4}$ 3460, 1758, 1732 cm^{-1}; NMR $\delta_{ppm}^{CDCl_3}$ methylene group of 2, 3-pyrazoline ring at 2.62 and 3.00 (m, 2H, J=18.0, 2.0 Hz), 3.20 (s, 1H, $-\underline{CH}-COOCH_3$), 5.53 (bs, 1H, $-\underline{NH}-N=CH-$), 6.51 (bs, 1H, $-CH_2-\underline{CH}=N-$), Found: C, 55.66; H, 6.01; N, 11.53%. Calcd. for $C_{11}H_{14}O_4N_2$: C, 55.45; H, 5.92; N, 11.76%.

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