The best discriminant function formulated within 100 times of iterative calculation was Eq. 5 which was obtained after 10 times of adaptation using Eq. 3 and 4 with $\alpha=2$.

$$L = -4.33 F_X - 2.46 V_{W-X} + 0.77 E_{S-Z} + 2.48 Y_{OMe} + 2.28 Y_{OH} + 1.42$$
$$n = 16, \quad \% \text{ correct} = 93.8 \qquad (5)$$

Goodness of fit for this classification was highly significant at the 0.001 level by the χ^2 test.[8] Equation 5 indicates that electron-donating substituent X, and OMe or OH (OMe being a slightly better) for substituent Y enhance the activity against solid sarcoma 180, whereas bulky substituents X and Z causing large steric hindrance reduce the effectiveness.

Besides the ALS method, three classification procedures were tested with the same structure-activity data for the purpose of comparison: the simple least-squares method[9] (5), the Rao-type discriminant analysis[10] (4), and the K-nearest neighbor method[11] with $K=1$ (7). The figures in the parentheses after the methods were the number of molecules misclassified. The best results were obtained by the ALS method: Only one compound was uncorrectly assigned.

In conclusion, the results of this study show that the ALS classification is effective for relating structure to activity rating of compounds. It is suggested that the ALS method can aid significantly in rational design of more potent drugs for cancer chemotherapy and other categories.

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Structure of Stephanthraniline A

A new polyoxypregnane derivative, stephanthraniline A, was isolated from the stem of *Stephanotis japonica* Makino (Asclepiadaceae), and its structure was determined.

Keywords——*Stephanotis japonica* Makino; Asclepiadaceae; polyoxypregnane; stephanthraniline A; sarcostin; ester-linkage; ^{13}C-NMR

In an earlier investigation of constituents of *Stephanotis japonica* Makino (Asclepiadaceae), C/D-*cis*-polyoxypregnane, sarcostin, lineolon, deacylmetaplexigenin, and stephanol were isolated.[1] In this communication, we wish to describe the isolation and structure of a new compound from the same source. The aglycone mixture, obtained after a mild acid hydrolysis of the crude glycoside, was separated by silica gel column chromatography and preparative

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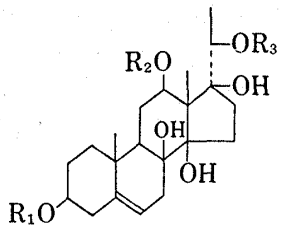
TLC. These procedures yielded a new ester type aglycone, stephanthraniline A, as a major component.



I: R_1=H, R_2=-C(=O)-C$_6$H$_4$-NH-CH$_3$, R_3=Ac

II: R_1=R_2=R_3=H

III: R_1=R_2=H, R_3=-C(=O)-C$_6$H$_4$-NH-CH$_3$

IV: R_1=H, R_2=-C(=O)-CH=CH-C$_6$H$_4$-, R_3=-C(=O)-C$_5$H$_4$N

VII: R_1=Ac, R_2=R_3=H

VIII: R_2=Ac, R_2=H, R_3=-C(=O)-C$_6$H$_5$

IX: R_1=Ac, R_2=-C(=O)-C$_6$H$_5$, R_3=H

X: R_1=R_3=Ac, R_2=-C(=O)-C$_6$H$_5$

XI: R_1=R_2=Ac, R_3=-C(=O)-C$_6$H$_5$

XII: R_1=R_2=Ac, R_3=H

XIII: R_1=R_3=Ac, R_2=-C(=O)-CH=CH-C$_6$H$_5$

XIV: R_1=R_3=Ac, R_2=-C(=O)-C$_6$H$_4$-NH-CH$_3$

Chart 1

Stephanthraniline A (I) (Chart 1) shows the following data: mp 170—173° (from acetone–hexane), positive to the Liebermann–Burchard reaction, purple to blue with $SbCl_3$ reagent, positive to the Dragendorff reagent, negative to the Keller–Killiani reaction; $[\alpha]_D$+17.9° (c=0.089 in $CHCl_3$); *Anal.* Calcd. for $C_{31}H_{43}NO_8$: C, 66.76; H, 7.77; N, 2.51. Found: C, 66.98; H, 7.85; N, 2.42; ultraviolet (UV) λ_{max}^{EtOH} nm: 222 (ε 35840), 253 (ε 11200); infrared (IR) ν_{max}^{Nujol} cm^{-1}: 3370, 1730, 1705, 1670, 1575; nuclear magnetic resonance (NMR) ($CDCl_3$) δ: 1.13 (19-CH_3), 1.31 (d, J=6 Hz, 21-CH_3), 1.52 (18-CH_3), 1.92 (*O*-acetyl), 2.90 (d, J=6 Hz, NH-CH_3), 3.52 (1H, m, 3α-H), 4.64 (1H, d.d, J=6, 11 Hz, 12α-H), 4.79 (1H, q, J=6 Hz, 20-H), 5.38 (1H, m, 6-H), 6.65 (2H, m), 7.40 (1H, m), 7.73 (1H, m, -NH), 7.86 (1H, m); MS *m/e*: 557 (M^+), 346, 151 (N-methylanthranilic acid, base peak). The area to which N-methylanthranilic acid moiety is attributed in the NMR spectrum of I was identical with that of the authentic sample. Hydrolysis of I with 5% methanolic KOH gave a neutral product, mp 147—152°, which was identified with sarcostin (II) from comparison of NMR, IR, and mass spectra. From these data, it is assumed that I is an N-methylanthraniloyl and acetyl ester of sarcostin.

Mild alkaline hydrolysis (K_2CO_3 in MeOH) of stephanthraniline A **(I)** gave a monoester (III), mp 161—164.5°. This compound exhibited the following spectroscopic properties: UV λ_{max}^{EtOH} nm 221 (ε 36300), 254 (ε 10900); IR ν_{max}^{Nujol} cm^{-1} 3350, 1690, 1665, 1580, 1440; MS *m/e*: 515 (M^+), 449 (M^+-H_2O), 151 (N-methylanthranilic acid, base peak); NMR ($CDCl_3$) δ: 1.13 (19-CH_3), 1.35 (d, J=7 Hz, 21-CH_3), 1.38 (18-CH_3), 2.87 (N-CH_3), 3.48 (d.d, J=6, 11 Hz, 12α-H), 3.50 (m, 3α-H), 5.33 (m, 6-H), 5.37 (q, J=6 Hz, 20-H), 6.55 (2H, m), 7.36 (1H, m), 7.79 (1H, m).

From the chemical shifts and coupling patterns in the NMR spectrum and the fact that acetylation of III was not entirely successful, this monoester would be represented by the structure III. However, the formation of only III on mild alkaline hydrolysis of I does not

show that *O*,N-methylanthraniloyl group is present at C-20 of I, because acyl group migration from C-12 to C-20 has been observed on same condition in study of structure of gagaminin (IV).[2)]

Compared with NMR spectrum of diester type aglycone, where structure has previously been established, for example, 12β-*O*-tigloyl-20-*O*-acetylutendin (12α-H, 4.63 ppm, 20-H, 4.65 ppm) (V), 12β-*O*-acetyl-20-*O*-tigloylutendin (12α-H, 4.62 ppm, 20-H, 5.30 ppm) (VI) (Chart 2), it is reasonable that N-methylanthraniloyl group is present at C-12 in I (12α-H, 4.64 ppm,

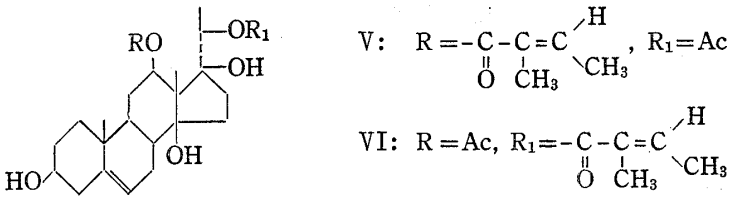


Chart 2

20-H, 4.79 ppm). Attempt has been made to determine the location of ester-linkage by the use of ^{13}C–NMR spectrum.

Treatment of 3β-*O*-acetylsarcostin (VII) with 1.2 molar benzoyl chloride in pyridine gave a mixture of 12β-benzoate and 20-benzoate. The product was separated by preparative TLC, and 3β-*O*-acetyl-20-*O*-benzoylsarcostin (VIII) and 3β-*O*-acetyl-12β-*O*-benzoylsarcostin (IX) were obtained in the approximate ratio of 1:1. Acetylation of the latter with acetic anhydride at 60° in pyridine yielded an acetate (X). Compound (XI) with reversed location of the ester linkage was obtained by benzoylation of 3β,12β-di-*O*-acetylsarcostin (XII).

^{13}C–NMR spectra of two benzoates (X and XI) were recorded on JEOL JNM-FX 100 pulsed Fourier transform NMR spectrometer. Chemical shifts have been assigned on the

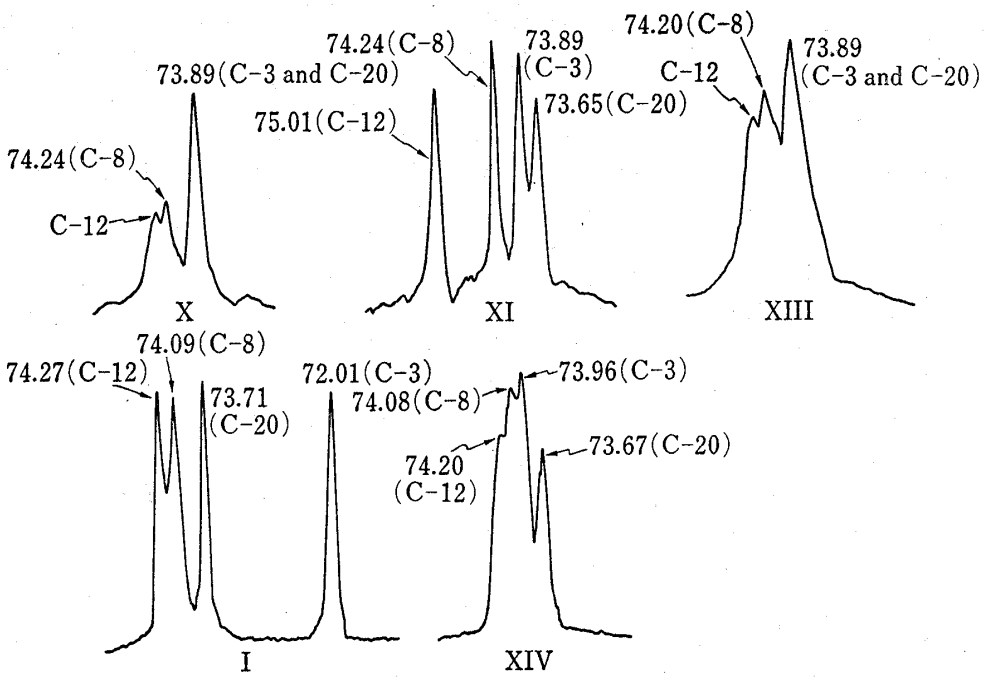


Fig. 1. NMR Spectra were determined in $CDCl_3$ Solution using Tetramethylsilane as an Internal Standard

2) T. Yamagishi, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 2289 (1972).

basis of off-resonance experiments and comparison among the related compounds.[3] Spectral pattern and chemical shifts of C-3, C-8, C-12, and C-20 are shown in Fig. 1. The spectrum of penupogenin diacetate (XIII) is similar to that of X. These results showed the possibility of the use of ^{13}C–NMR for determination of the ester linkage site in esterified sarcostin with aromatic carboxylic acid and acetic acid. The spectra of stephanthraniline A and its acetate (XIV) are depicted in Fig. 1, and are similar to those of 3β,20-di-*O*-acetyl-12β-*O*-benzoylsarcostin (X) and penupogenin diacetate (XIII). From these evidences, the structure of stephanthranilin A was determined as 12β-*O*,N-methylanthraniloyl-20-*O*-acetylsarcostin (I).

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