Journal of Natural Products
Vol. 47, No. 1, pp. 47-51, Jan-Feb 1984

STUDIES ON HETEROCYCLIC COMPOUNDS, V.[1] SYNTHETIC INVESTIGATION OF GLYCARPINE

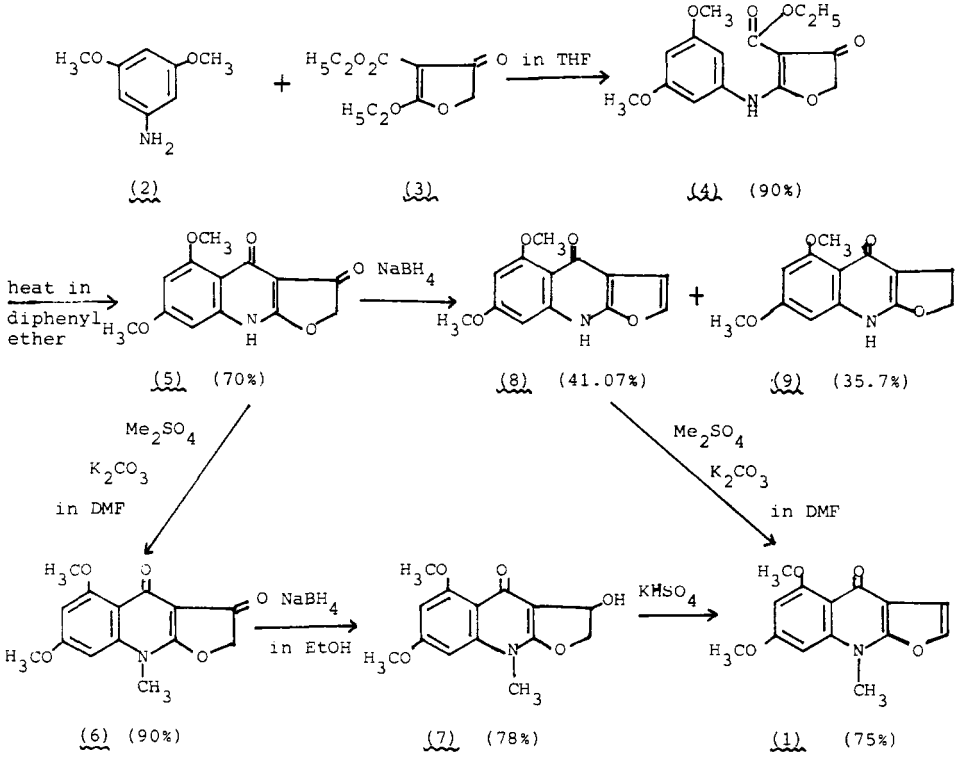
SHENG-CHU KUO, TSUNG-PING LIN,
LONG-DAR LIN, HONG-YEN HSU, and CHUN-HSIUNG WU

School of Pharmacy, China Medical College
Taichung 400, Taiwan, Republic of China

ABSTRACT.—In order to examine the biological activities of furo(2,3-*b*)quinolin-4-one derivatives, a synthetic approach to the structure reported for glycarpine from *Glycosmis cyanocarpa* was unambiguously carried out. The spectral data of the synthetic product were not identical with those described previously for glycarpine; thus, the original structure of this alkaloid should be revised.

The furoquinoline alkaloids are well known in nature and occur almost exclusively in the Rutaceae with only few exceptions. Some of these alkaloids have been found to possess some interesting pharmacological activities (2).

Recently, glycarpine, a novel furo(2,3-*b*)quinolin-4-one alkaloid, was isolated from *Glycosmis cyanocarpa* Spreng. (Rutaceae) by Sarkar and co-workers (3). The structure of glycarpine was suggested to be 5,7-dimethoxy-9-methyl-4,9-dihydrofuro(2,3-*b*)quinolin-4-one (**1**) based on the spectral evidence. Some furo(2,3-*b*)quinolin-4-one compounds have been synthesized by the method of Tuppy and Böhm (4). However, the synthesis of glycarpine has not been reported. In this work, we attempted to prepare the reported structure of glycarpine (**1**) by the modified Tuppy and Böhm method (Scheme 1).



SCHEME 1

[1]For Part IV, see reference (1).

RESULTS AND DISCUSSION

3,5-Dimethoxyaniline (**2**) was condensed with ethyl 2-ethoxy-4-oxo-4,5-dihydrofuran-3-carboxylate (**3**) (prepared *in situ* from chloroacetyl chloride and ethyl sodiomalonate) to afford the tetronic ester, ethyl 2-(3,5-dimethoxyanilino)-4-opxo-4,5-dihydrofuran-3-carboxylated (**4**). Thermal cyclization of **4** in boiling diphenyl ether gave 5,7-dimethoxy-2,3,4,9-tetrahydro(2,3-*b*)-quinolin-3,4-dione (**5**). Compound **5** was methylated with dimethyl sulfate and K_2CO_3 in DMF to afford compound **6** with formula $C_{14}H_{13}NO_5$. The ir spectrum of **6** showed two carbonyl absorptions at 1700 and 1640 cm^{-1}. Its ^{1}H-nmr (CF_3COOD) spectrum exhibited three methyl groups at δ 4.05 (3H, s, N-CH_3), δ 4.13 (3H, s, 7-OCH_3), and δ 4.30 (3H, s, 5-OCH_3). A methylene group was found at δ 5.25 (2H, s, -CH_2-), and a multiplet at δ 6.94 (2H, m) was assigned to the aromatic protons. Based on the spectral data, the structure of **6** was assigned as 5,7-dimethoxy-9-methyl-2,3,4,9-tetrahydrofuro(2,3-*b*)quinolin-3,4-dione. Reduction of **6** with excess sodium borohydride in ethanolic alkali afforded the corresponding alcohol, 3-hydroxy-5,7-dimethoxy-9-methyl-2,3,4,9-tetrahydrofuro(2,3-*b*)quinolin-4-one (**7**). Compound **7** was then dehydrated with anhydrous $KHSO_4$ or concentrated H_2SO_4 to afford crystals of **1** with mp at 170°. The mass spectrum (M^+ 259) and elemental analysis of **1** suggested a molecular formula of $C_{14}H_{13}NO_4 \cdot H_2O$. The uv spectrum showed λ max (EtOH) at 253.5, 333 nm (log 4.45, 4.01), which is very similar to that of isoacronycidine (2). The ir spectrum showed a carbonyl absorption at 1640 cm^{-1}, and the ^{1}H-nmr spectrum ($CDCl_3$) exhibited three methyl groups at δ 3.74 (3H, s, N-CH_3), δ 3.86 (3H, s, 7-OCH_3), and δ 3.89 (3H, s, 5-OCH_3). Two protons on the aromatic ring were found at δ 6.33 (2H, s), and AB quartets were found at δ 6.91 (H, d, J=2.5 Hz) and δ 7.14 (H, d, J=2.5 Hz), representing the protons at position 2 and 3 of the furan ring.

Based on the above data, the structure of **1** was determined to be 5,7-dimethoxy-9-methyl-4,9-dihydrofuro(2,3-*b*)quinolin-4-one. Tentative assignments for the ^{13}C-nmr spectrum of **1** (Table 1) were made by analogy with model compounds furoquinoline alkaloids (6), quinolin-4-one compounds (7), and benzofuran compounds. Of the two quinolin-4-one tertiary carbon signals, carbon 6 was assigned to that at 92.40 ppm because of its proximity to two -OCH_3 groups, and the lower field 97.03 ppm signal was assigned to carbon 8. Signals for carbons 3a and 4a and 7 and 9a were assigned by virtue of their long-range coupling in the gated spectrum.

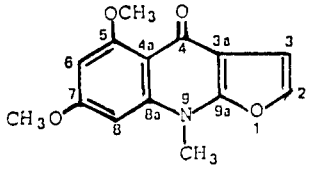
The spectral data of the synthetic product (**1**) were not identical with those described previously for glycarpine (3). In order to confirm the structure of **1**, the method of Govindachari (2), which involves the treatment of **5** with excess sodium borohydride and ethanolic alkali, was employed to prepare **1**. The usual work-up yielded a mixture of two products.

After chromatography over silica gel and repeated crystallization, two pure compounds, **8** and **9**, were isolated. The ^{1}H-nmr spectrum of **8**, having signals at δ 3.87 (3H, s, 7-OCH_3), δ 4.03 (3H, s, 5-OCH_3), δ 6.35 (H, d, J=2.5 Hz, 6-H), δ 6.81 (H, d, J=2.5 Hz, 8-H), δ6.91 (H, d, J=2.5 Hz, 3-H), δ 7.42 (H, d, J=2.5 Hz, 2-H), and δ 9.86 (H, b, -NH), was consistent with the structure 5,7-dimethoxy-4,9-dihydrofuro(2,3-*b*)quinolin-4-one. The ^{1}H-nmr spectrum of **9**, having signals at δ 3.20 (2H, t, J=11.0 Hz, -OCH_2-CH_2-), δ 3.85 (3H, s, 7-OCH_3), δ 3.96 (3H, s, 5-OCH_3), δ 4.67 (2H, t, J=11.0 Hz, -O-CH_2-CH_2-), δ 6.28 (H, d, J=2.5 Hz, 6-H), and δ 6.75 (H, d, J=2.5 Hz, 8-H), suggested compound **9** to be 5,7-dimethoxy-2,3,4,9-tetrahydrofuro-(2,3-*b*)quinolin-4-one. Methylation of **8** with dimethyl sulfate and K_2CO_3 in DMF yielded *N*-methyl derivative which was demostrated to be the same as the dehydration product of **7**. With all of the preceding data, the structure of the synthetic product (**1**) appeared to be well established, indicating that the natural product

was formulated incorrectly in the original publication (3), and thus, the original structure of glycarpine should be revised. As to the structure of glycarpine isolated from *G. cyanocarpa* (3), it seems difficult to suggest a structure with a carbonyl group on furoquinolin-4-one skeleton, based upon the published spectral data, especially the absorption at 1720 cm^{-1} in the ir spectrum.

From this study, 5,7-dimethoxy-9-methyl-4,9-dihydrofuro(2,3-*b*)quinolin-4-one (**1**) was synthesized. The screening test of its pharmacological activities is still in progress, and results will be published later.

TABLE 1. ^{13}C-nmr Chemical Shifts of 5,7-Dimethoxy-9-methyl-4,9-dihydrofuro(2,3-*b*)quinolin-4-one (**1**)[a]



ppm	Multiplicity	Carbon
34.75	q	N-CH_3
57.71	q	O-CH_3
57.95	q	O-CH_3
92.40	d	C-6
97.03	d	C-8
108.57	s	C-3a
109.22	d	C-3
109.92	s	C-4a
141.85	d	C-2
144.20	s	C-8a
157.38	s	C-5
163.83	s	C-7
166.50	s	C-9a
173.61	s	C-4

[a]JEOL FX-100 TMS as an internal standard; CD_3COOD as a solvent.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined in open-ended capillary tubes on a Thomas Hoover apparatus and are uncorrected. Ir spectra were taken in KBr using a Shimadzu-IR-440. The ^{1}H-nmr spectra were recorded in $CDCl_3$, unless otherwise indicated, on a JEOL-PMX 60 spectrometer. The ^{13}C-nmr spectra were recorded in CD_3COOD on a JEOL-FX 100 NMR spectrometer. TMS was used as internal standard. Mass spectra were determined on Hitachi RMU 71 mass spectrometer. Tlc was carried out on Wakogel B-5FM plates.

ETHYL 2-(3,5-DIMETHOXYANILINO)-4-OXO-4,5-DIHYDROFURAN-3-CARBOXYLATE (**4**).—Sodium hydride (2.0 g), previously washed with dry *n*-hexane, was suspended in dry THF (100 ml) and added slowly, with shaking, over 10 min to a solution of diethyl malonate (13.7 ml) in dry THF (30 ml). The reaction mixture was refluxed on a water bath for 2 min, then cooled to 10-12° and chloroacetyl chloride (13.7 ml) in dry THF (20 ml) was added dropwise over 10 min. The solution was kept at this temperature for 1 h, at 40-45° for another hour and 3,5-dimethoxyaniline (5.0 g) in dry THF (50 ml) was then added dropwise over 20 min. The reaction mixture was left at room temperature overnight, heated under reflux for 1 h, then cooled and poured into ice H_2O. The precipitated solid was extracted with $CHCl_3$ and the extract was washed with 1 N NaOH solution (400 ml). The alkaline solution was neutralized with concentrated HCl and reextracted with $CHCl_3$. The organic layer was washed with H_2O and dried ($MgSO_4$). The solvent was partially evaporated and the concentrated residue refrigerated for 2 days. The precipitate was collected and recrystallized from EtOH to afford the tetronic ester (4), 8.6 g (90%); mp 136-138°; ir ν max (KBr) 3260 (NH), 1700 (-$COOC_2H_5$), 1640, 1590, and 1560 cm^{-1}; uv λ max (MeOH) 299; ^{1}H-nmr δ

1.38 (3H, t, $-CH_2-CH_3$), 3.80 (6H, s, $-OCH_3$), 4.35 (2H, q, $-CH_2-CH_3$), 65.3 (H, s, 4-H), 6.4-6.5 (2H, 2-H, 6-H) and 10.22 (H, s, -NH). Calcd for $C_{15}H_{17}NO_6$; C 58.63, H 5.53, N 4.56. Found; C 58.60, H 5.52, N 4.67%.

5,7-DIMETHOXY-2,3,4,9-TETRAHYDROFURO(2,3-*B*)QUINOLIN-3,4-DIONE (**5**).—The tetronic ester (**4**, 4.0 g) as a fine powder was added with stirring in one lot to diphenyl ether (35 ml) maintained at 240°. The temperature was then raised to 256° and kept there for 5 min. The mixture was cooled to room temperature and diluted with a large volume of hexane to precipitate a dark solid that was collected and washed with hot hexane and purified by chromatography on silica gel (100 g) column. Elution with $CHCl_3$-MeOH (9:1) yielded a light-brown solid of 3-oxofuroquinolone (**5**), 2.55 g (70%); mp 232-234°; ir ν max (KBr) 4260 (NH), 1720 ($-O-CH_2-CO-$), 1660, 1640 (Ar-CO-C=C-), 1590, and 1550 cm^{-1}; uv λ max (MeOH) 257.5 and 318.5 nm; ^{1}H-nmr δ (CF_3COOD) 4.1 (3H, s, 7-OCH_3), 4.3 (3H, s, 5-OCH_3), 5.23 (2H, s, $-OCH_2-CO-$), 6.86 (H, s, 8-H), and 7.03 (1H, s, 6-H). Calcd for $C_{13}H_{11}NO_5$; C 59.77, H 4.21. Found; C 59.01, 59.50; H 4.27, 4.30%.

5,7-DIMETHOXY-4,9-DIHYDROFURO(2,3-*B*)QUINOLIN-4-ONE (**8**) AND 5,7-DIMETHOXY-2,3,4,9-TETRAHYDROFURO(2,3-*B*)QUINOLIN-4-ONE (**9**).—The 3-oxofuroquinolone (**5**, 0.6 g), suspended in a mixture of EtOH (150 ml) and 2 N NaOH solution (20 ml), was treated portionwise with excess of sodium borohydride (7.5 g) over 1 h. The mixture was refluxed for 6 h until a solid separated. It was then filtered, and the filtrate was concentrated *in vacuo*. The residues were dissolved in H_2O, and the combined aqueous solution was filtered; the filtrate was nautralized with glacial HOAc and refrigerated for 2 days. The precipitated brown solid was collected and dissolved in $CHCl_3$, washed with H_2O, dried ($MgSO_4$, and evaporated. The residue of **8** and **9** after evaporation was subjected to chromatography over silica gel (50 g) and eluted with $CHCl_3$-4%-MeOH. Removal of the solvent from the eluate yielded a brown residue that was repeatedly crystallized from $CHCl_3$-hexane to yield compound **8** (0.23 g, 41.07%); mp 188-189°; ir ν max (HBr) 3280 (NH), 1660, 1630, and 1610 cm^{-1}; uv λ max (MeOH) 250.5 and 323 nm; ^{1}H nmr δ ($CDCl_3$) 3.87 (3H, s, 7-OCH_3), 4.03 (3H, s, 5-OCH_3), 6.35 (1H, d, J=2.5 Hz, 6-H), 6.81 (1H, d, J=2.5 Hz, 8-H), 6.91 (H, d, J=2.5 Hz, 3-H), 7.42 (H, d, J=2.5 Hz, 2-H), and 9.86 (H, b, NH). Calcd for $C_{13}H_{11}NO_4$; C 63.67, H 4.48. Found: C 64.01, 64.10; H 4.40, 4.45, and compound **9** (0.20 g, 35.7%); mp 191°; ir ν max (KBr) 3250 (NH), 1710, 1640 and 1610 cm^{-1}; uv λ max (MeOH) 258 and 319 nm; ^{1}h-nmr δ ($CDCl_3$) 3.20 (2H, t, J=11.0 Hz, $-OCH_2-CH_2-$), 3.85 (3H, s, 7-OCH OCH_3), 3.96 (3H, s, 5-OCH_3), 4.67 (2H, t, J=11.0 Hz, $-OCH_2-CH_2-$), 6.28 (H, d, J=2.5 Hz, 6-H) and 6.75 (h, d, J=2.5 Hz, 8-H). Calcd for $C_{13}H_{13}NO_4$; C 63.15, H 5.22. Found; C 63.20, H 5.23%.

5,7-DIMETHOXY-9-METHYL-2,3,4,9-TETRAHYDROFURO(2,3-*B*)QUINOLIN-3,4-DIONE (**6**).—The cyclized product (**5**) (3.0 g) was suspended in DMF (20 ml) and warmed to 40°. To the suspension was added anhydrous K_2CO_3 (25.0 g). Dimethyl sulfate (20.0 g) was then added dropwise over 1 h. The reaction mixture was filtered and the precipitate washed with $CHCl_3$. The filtrate and washings were combined, and the solvent was evaporated *in vacuo*. Ice H_2O was added to the residue, and the precipitate was collected by filtration, washed with water, and purified by chromatography on silica gel (100 g) column. Elution with $CHCl_3$ yielded colorless crystals **6** (2.84 g, 90%); mp 254-258°; ir ν max (KBr) 1700, 1640, 1600, 1570 and 1540 cm^{-1}; uv λ max (MeOH) 257.5- and 318.5 nm; ^{1}H-nmr δ (CF_3COOD) 4.05 (3H, s, N-CH_3), 4.13 (3H, s, 7-OCH_3), 4.30 (3H, s, 5-OCH_3), 5.25 (2H, s, $-CH_2-$), and 6.94 (2H, m, 6-H, 8-H). Calcd for $C_{14}H_{13}NO_5$; C 59.77, H 4.21. Found: C 60.01, 60.20; H 4.25, 4.28%.

3-HYDROXY-5,7-DIMETHOXY-9-METHYL-2,3,4,9-TETRAHYDROFURO (2,3-*B*) QUINOLIN-4-ONE (**7**).—A solution of compound **6** (0.4 g) in EtOH (160 Ml) was cooled to 15° and treated with sodium borohydride (2.0 g) over a period of 1 h. The resulting yellow solution was left at room temperature until it became colorless (3 h). The solvents were removed *in vacuo*, and the residue was extracted with $CHCl_3$, dried, and concentrated. Recrystallization from $CHCl_3$-MeOH yielded the alcohol **7** (0.314 g, 78%), mp 171-173°; ir ν max (KBr) 3100 (CH-OH), 1640, 1610, 1580, 1530 and 1510 cm^{-1}; ^{1}H-nmr δ (CF_3COOD) 4.13 (3H, s, N-CH_3), 4.33 (6H, s, 5- and 7-OCH_3), 6.98 (2H, s, 6-H, 8-H), 7.2 (1H, d, J=2.5 Hz, 3-H) and 7.72 (1H, d, J=2.5 Hz, 2-H). Calcd for $C_{14}H_{15}NO_5$; C 60.64, H 5.41. Found: C 60.10, 60.40; H 5.45, 5.48%.

5,7-DIMETHOXY-9-METHYL-4,9-DIHYDROFURO(2,3-*B*)QUINOLIN-4-ONE (**1**).—A solution of compound **7** (0.15 g) in dry dioxan was refluxed with freshly fused K_2SO_4 (0.225 g). After 2 h, additional K_2SO_4 (0.375 g) was added, and refluxing was continued for 4 h. The hot dioxan solution was filtered, and the solvent was removed *in vacuo*. The yellow residue was purified by chromatography over silica gel (50 g) eluting with $CHCl_3$. The eluate was evaporated and the residue crystallized from $CHCl_3$-n-hexane to give white crystalline needles of **1** (0.10 g, 75%); mp 171°; ir ν max (KBr) 1640, 1620, 1590, 1540 and 1520 cm^{-1}; ms *m/z* 259 (M^+); uv λ max (MeOH) 253.5, 333 nm; ^{1}H-nmr δ ($CDCl_3$) 3.74 (3H, s, N-CH_3), 3.86 (3H, s, 7-OCH_3), 3.89 (3H, s, 5-OCH_3), 6.33 (2H, s, 6-H, 8-H) 6.91 (H, d, J=2.5 Hz, 3-H), and 7.14 (H, d, J=2.5 Hz, 2-H). Calcd for $C_{14}H_{13}NO_4$; C 64.86, H 5.02. Found: C 64.85, 64.87; H 5.05, 5.03%.

In an alternate preparation, concentrated H_2SO_4 was added dropwise to **7** (0.15 g) until it was completely dissolved and the solution was neutralized with $NaHCO_3$. The mixture after neutralization was extracted with $CHCl_3$, and the solvent removed *in vacuo*. The residue was crystallized from $CHCl_3$-*n*-hexane to give the same compound **1**.

N-METHYLATION OF **8**.—A suspension of compound **8** (0.3 g) in DMF (25 ml) was warmed to 40° and anhydrous K_2CO_3 (5.0 g) was then added. Dimethyl sulfate (3.0 g) was added dropwise over 1 h, and the precipitate was filtered off and washed with $CHCl_3$. The filtrate and washings were combined, and the solvent was evaporated *in vacuo*. Ice H_2O was added to the residue, and the mixture was filtered. The recipitate was washed with H_2O and purified by chromatography on silica gel (50.0 g). Elution with $CHCl_3$ yielded colorless crystals of **1** (0.285 g, 90%).

ACKNOWLEDGMENTS

The authors wish to thank the National Science Council of the Republic of China for its financial support.

LITERATURE CITED

1. S. Kuo, C. Wu, and C. Wang, *Heterocycles*, **16**, 231 (1981).
2. T.R. Govindachari, S. Prabhaker, V.N. Ramachan, and B.R. Pai, *Indian J. Chem.*, **9**, 1031 (1971).
3. M. Sarker, S. Kundu, and D.P. Chakraborty, *Phytochemistry*, **17**, 2145 (1978).
4. S. Prabhaker, B.R. Pai, and V.N. Ramachandran, *Indian J. Chem.*, **9**, 191 (1971).
5. J.W. Huffman, S.P. Gara, and J.H. Cuei, *J. Org. Chem.*, **31**, 1276 (1966).
6. J.F. Ayafor, B.L. Sondergam, A.N. Bilon, E. Tsamo, S.F. Kimbu, and J.I. Okogum, *J. Nat. Prod.*, **45**, 714 (1982).
7. P.A. Claret and A.G. Osborne, *Org. Magn. Reson.*, **10**, 35 (1977).
8. L. Mester and M. Jonescu, *Phytochemistry*, **10**, 2205 (1971).

Received 26 April 1982